

**The Effect Endurance Training has on
Resting Metabolic Rate, Heart Rate, and Respiratory Exchange Ratio
Variability**

A Dissertation

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Dedication

I dedicate this to my father V. Terry Rhodes M.D., my mother Sharon Rhodes, and my life partner Devon Hutton M.D. Without their support and patience, I would never have accomplished this achievement.

Abstract

Technology advances provide exercise and sport physiologist real-time data allowing for precision in assessing the interaction between external stimuli and physiological responses. The results of these advances provide valuable data points specific to clinical disease as well as performance measures. This increase in data points as well as the scientific and medical interpretation has provided improved insight specific to the interactions of training interventions and their influence on an individual's physiology. The aim of this study was to utilize the method of variability analysis to assess the effect of a 16-week endurance running training program has on resting metabolic rate, substrate utilization, and heart rate variability in college-aged runners preparing for their first marathon. A sample population of 17 of the total 106 recreational marathoners who were enrolled in a Marathon Training course at the University of Minnesota volunteered to participate in this specific research. All participants completed two lab testing sessions that consisted of three visits over the course of 5 months. These visits consisted of a 2-mile time trial on an indoor track, assessment of body composition and aerobic capacity, and a resting metabolic test. The first period of testing was completed in early December prior to the start of the training program. The second period of testing was during the final two weeks of training before all participants ran the same marathon. Data collected from all visits was examined for changes pre- to post-training. Time series data (heart rate and respiratory exchange ratio) was analyzed using the non-linear variability analysis method of sample entropy. Participants in this study showed increases in fat oxidation during submaximal steady-state running ($p < 0.001$) as well as increased running economy ($p < 0.001$). However, metabolic and heart rate changes were not observed at rest ($p = 0.915$). Analysis of metabolic and heart rate data using both linear and non-linear methods provided insight into the effect that training for a running marathon has on human physiology. Additionally, more in-depth methods of analysis have increased the level in which individual variations in adaptations can be identified.

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Chapter 1: Introduction

Chapter 1: Introduction:

Obesity and the comorbidities associated with it, such as hypertension, diabetes, hyperlipidemia, and hypercholesterolemia, are an epidemic issue worldwide (Campbell, Wallman, & Green, 2010). Overeating alone does not explain the dramatic increase in the prevalence in obesity and metabolic disorders. Additionally, the Surgeon General suggests that significant health benefits can be obtained by including a moderate amount of physical activity on most days of the week (U.S. Department of Health and Human Services, 1996). Exercise and the effects it has on metabolism have been examined extensively to identify the role exercise can play in helping correct this world epidemic in obesity (Dolezal & Potteiger, 1998; Lee, Sedlock, Flynn, & Kamimori, 2009).

1.1 Background

Metabolism is the sum of all processes that convert chemical energy into other forms of energy within the body. Metabolism provides the energy for all types of cellular work: mechanical, synthetic, chemical, osmotic, and electrical (Weir, 1949). It is this conversion of potential energy into chemical energy that sustains life as well as allows for sustained movement and exercise. Small but chronic changes in the total daily energy expenditure and specifically the resting metabolic rate (RMR), which comprises approximately 60-70% of the total daily energy expenditure in humans, have a cumulative impact on the maintenance of body mass (Poehlman, Melby, & Bradylak, 1988). The role in which exercise influences RMR has been investigated with mixed results stemming from varied

types of exercise as the total volume and the intensity of the exercise (Gilliat-Wimberly, Manore, Woolf, Swan, & Carroll, 2001; Sullo, Cardinale, Brizzi, Fabri, & Maffulli, 2004; Trembley, Fontaine, Poehlman, Mitchell, Perron, & Bouchard, 1986; Lemmer et al., 2001; Morio et al., 1998; Poehlman & Danforth, 1991; Wilmore et al., 1998; Lee, Sedlock, Flynn, & Kamimori, 2009; Campbell et al., 2010; Molé, 1990).

In addition to the overall metabolic rate, measuring substrate utilization is another way to investigate how metabolism is affected by exercise. Substrate utilization can be determined by measuring the respiratory exchange ratio (RER) using breath-to-breath analysis (Kelley, Goodpaster, Wing, & Simoneau, 1999). It has been observed that some obese individuals, utilize carbohydrates as the primary energy substrate and show little variation in substrate utilization during physical activity or during rest (Goodpaster, Wolfe, & Kelley, 2002). This state has been described as metabolic inflexibility and is characterized by a similar ratio of carbohydrate to fat oxidation independent of the feeding or fasting state (Corpeleijn, Saris, & Blaak, 2009). The influence of exercise on altering substrate utilization in trained and untrained individuals can provide insight into types of interventions that have the potential altering obesity and other symptoms of metabolic syndrome (Barwell, Malkova, Leggate, & Gill, 2009; Snyder, Donnelly, Jakobsen, Hertner, & Jakicic, 1997).

Many physiological systems in the human body that control metabolism and how the human body responds to exercise appear to be rhythmic patterns,

yet have underlining complexity. Traditional linear calculations of mean RMR, RER, and heart rate have provided limited and mixed results regarding the affect of exercise on metabolism at resting and during steady-state exercise (Trembley, Fontaine, Poehlman, Mitchell, Perron, & Bouchard, 1986; Lee, Sedlock, Flynn, & Kamimori, 2009; Goedecke, Gibson, Grobler, Collins, Noakes, & Lambert, 2000; Blaak & Saris, 2002). These linear-based method of analyzing physiological data do not take into account the information that can be discovered when scale and variability of time series data is analyzed using non-linear variability techniques (Biltz, Unnithan, Brown, Marwood, Roche, Garrard, & Holloway, 2011). For example, the use of non-linear variability analysis techniques of heart rate variability (HRV) has provided information about how the autonomic nervous system adapts to exercise training (Mourrot, Bouhaddi, Perrey, Rouillon, & Regnard, 2004, Plews et al., 2012). The application of non-linear variability analysis techniques to variables of metabolism could provide a deeper understanding of the inherent complexity of human metabolism and valuable information about the effect exercise has on resting metabolism from both a clinical and sports performance perspective.

1.2 Summary and Research Objectives

To date, there is no research examining the affect of an exercise training intervention on non-linear variability of breath-to-breath RER during rest of athletes. Research specific to changes in RER and mean RER during rest after an aerobic/endurance based training intervention is mixed (Barwell et al., 2009;

Snyder et al., 1997; Broeder, Burrhus, Svanevik, & Wilmore, 1992a; Lee et al., 2009; Campbell et al., 2010). Observations during research within our lab, has indicated participants in a multi-year study on marathon training have variable changes in body weight and fat free mass after the training is completed (Brown, 2013). Additionally, Brown (2013) found significant increases in RER SampEn ($p < 0.05$) during steady state running upon the completion of a marathon training program.

Efforts to reverse the obesity epidemic have led to a wide variety of suggested interventions ranging from diet to varied exercise interventions. Understanding the interaction of specific modalities of exercise intervention and variations within, specific to the effect on metabolism and substrate utilization will allow for effective interventions to be implemented. Additionally, establishing non-invasive measurement and assessment tools can allow for individualization of such interventions when working with patients in clinical settings and athletes.

This research will address the following objectives:

1. Examine the effect of a 16-week marathon training program on resting metabolic rate in novice college-aged runners.
2. Evaluate changes in substrate utilization, using breath-to-breath respiratory exchange ratio, during steady state running and at rest following a 16-week marathon training program

3. Demonstrate the use of the non-linear variability analysis tool of Sample Entropy to evaluate variations of individual's heart rate and metabolic adaptations in substrate utilization following endurance run training

1.3 Significance

Previous studies have shown conflicting results regarding the effect that exercise training programs have on resting metabolic rate as well as changes in substrate utilization at rest and during exercise. This study will provide further information regarding the effect that endurance training has on metabolic adaptations by utilizing a training intervention which is greater than previous research as well as controlling for some overlooked variables during data collection. Additionally, by utilizing the novel method of non-linear variability analysis, the study may provide additional insight into what adaptations are occurring following endurance training interventions. The non-invasive nature of the methods used in this study will lay groundwork for clinicians as well as human performance experts of the interaction of fat mass, exercise, heart rate, and RER.

Chapter 2: Literature Review

Chapter 2: Literature Review

2.1 Basic Science background: Human Bioenergetics, Metabolism, and Measurement

Energy is required to sustain life. Humans obtain energy by utilizing potential energy stored within molecules via cellular respiration (Blei, Conly, & Kushmerick, 1993). This process results in the high-energy compound adenosine triphosphate (ATP). ATP is the energy currency used for a variety of biological work, including muscle contraction, synthesis of molecules, and transportation of substances. The hydrolysis of ATP releases the stored potential energy used by living cells to function. However, the body only stores limited amounts of ATP, approximately 7 to 8 $\mu\text{mol/g}$ (He et al., 2000). Most daily energy needs are met through the oxidation of carbohydrates and fats stored within the body, reserving stored ATP for short explosive bursts of energy (Blei et al., 1993).

Carbohydrates, fat and protein are the three energy-containing nutrients that are consumed. Macro-nutrients are stored as potential energy in the form of glycogen in muscle and the liver, as well as stored as triglycerides in muscle and adipose tissue (Green, 1991). The total amount of potential energy stored depends on the total number of calories consumed and the total calories expended (Blei et al., 1993).

Energy is stored in the body in two forms; fats in the form of triglycerides and carbohydrates in the form of glycogen in the muscle and liver (Green, 1991). Energy requirements are met by caloric consumption and the breakdown of

energy stores (Green, 1991). If no food is consumed for approximately seven hours, glucose will remain available at an appropriate level (Kang, 2008). The maintenance of blood glucose is achieved by increasing the use of fat and by mobilizing liver glycogen (Komi & Karlsson. 1978). Because glycogen stores are limited, oxidation of fat stores during the rested states is important. Protein is not stored as a primary energy source within the human body, it mainly serves as a structural component of muscle and organs (Rennie & Tipton, 2000). Therefore, the breakdown of protein to maintain glucose levels and hence provide energy can result in the loss of muscle and other lean tissue and is not ideal for health and athletic performance (Rennie & Tipton, 2000). Of the three energy-containing nutrients; carbohydrate, fat, and protein, the fat molecule has the largest quantity of energy per unit weight (Jeukendrup, Saris, & Wagenmakers, 1998). In a well-nourished individual at rest, breakdown of fat provides more than half of the total energy requirements (Kang, 2008). The potential energy stored in fat molecules of an 80kg male with 15% body fat equals 110,740 kcal. This is in contrast to the much smaller amount of stored potential energy of 2000 kcal stored as carbohydrate. Due to the large amounts of stored energy in the form of fats, during energy restriction, substantial amounts of fat are used to provide energy (Jeukendrup et al, 1998).

Through cellular respiration, the energy possessed by macronutrients is changed into chemical energy, which is then stored within energy substrates or converted to mechanical and heat energy (He et al., 2000). Stored energy exists

in a variety of chemical compounds, including ATP, phosphocreatine (PCr), glycogen, and triglycerides (Komi & Karlsson, 1978). As an energy currency, ATP can be readily used to meet immediate energy needs. However, this high-energy compound is stored in limited quantity. In fact, the body stores only approximately 7 to 8 $\mu\text{mol/g}$ body weight of ATP at any one time (He et al., 2000). This provides energy that can sustain maximal exercise, such as a 55 m sprint, the high and long jump, base running, and football play, for only several seconds (Komi & Karlsson, 1978). Consequently, in most sporting events and daily physical activities, ATP is replenished continuously through a series of chemical reactions involving energy transformation (Komi & Karlsson, 1978). Three distinct energy systems have been identified as playing a role in replenishing ATP: the ATP-PCr system, the glycolytic system, and the oxidative system (Green, 1991).

Most of the energy used daily comes from the oxidation of carbohydrate, lipid, and, in rare cases, protein consumed in the diet. This aerobic production of energy occurs through the oxidation of pyruvate which occurs within the mitochondria (Edwards, Margaria, & Dills, 1934). Researchers developed the method of indirect calorimetry as a non-invasive method to measure the level of metabolic oxidation that happens within the human body at rest and during exercise (Krough & Lindhard, 1920). Indirect calorimetry uses the concept that if the amount of oxygen taken in and amount of carbon dioxide expelled per breath is measured, information specific about what type and amount of fuel being

burned can be calculated. Through extensive research over several decades, scientists have shown that by collecting gases during human respiration assumptions can be made related to oxidative metabolism specific to muscle (Krough & Lindhard, 1920; Jeukendrup & Wallis, 2005). This measurement of respiratory gases is referred to as a metabolic measurement known as the respiratory exchange ratio (RER). When specifically measuring metabolism at the tissue level it is referred to as cellular respiration and measured as the respiratory quotient (RQ). Jeukendrup and Wallis (2005) demonstrated that at workloads below an individual's ventilator threshold that RQ and RER are equal.

When using breath-to-breath respiratory measurements to calculate RER the known amount of oxygen taken in is compared to the measured carbon dioxide expelled. The RER is simply the ratio of the carbon dioxide expelled to the oxygen consumed during each breath. Assumptions are made that when ATP used at a measured work rate comes from oxidative sources, ATP and creatine phosphate, stores remain constant, while protein catabolism is negligible, then the measured RER is equal to the RQ of the tissues within the system (Naimark, Wasserman, & McIlroy, 1964). These assumptions allow researchers to concur that at an RER of 0.70, 100% of the ATP being used by an individual comes from the oxidation of fats. An RER of 1.00 indicates that the used ATP is from carbohydrate oxidation. In addition to making assumptions about the composition of the oxidative metabolism, the amount of calories per liter of oxygen consumed

can also be calculated using tables and equations developed by Graham Lusk in 1910 (Lusk, 1923).

2.2 Resting metabolic rate and exercise:

Human metabolism is the collection of all anabolic and catabolic reactions that occur within the organism. The total energy expenditure of a human is the amount of energy used throughout a 24-hour period to sustain all these reactions. Total energy intake is regularly compared to total energy expenditure to identify net gains or losses in energy over time. Net gains in energy are associated with weight gain, whereas net energy losses are associated with weight loss. Total daily energy expenditure can be broken down into three components; resting or basal metabolic rate, thermic effect of food (TEF), and energy expenditure during physical activities.

Resting metabolic rate (RMR) may be viewed as the minimal rate of metabolism necessary to sustain critical body functions, such as the energy for a variety of cellular events that are essential to the life of an organism. Resting metabolic rate constitutes about 60% to 75% of the total daily energy expenditure and therefore represents the largest component of daily energy expenditure (Broeder, Burrus, Svanevik, & Wilmore, 1992a). Due to the large role that RMR plays in daily energy expenditure, investigators have looked at alterations in RMR when looking into weight management interventions. For individuals to lose weight a negative energy balance needs to exist in which daily energy expenditure exceeds daily energy intake (Lee et al., 2009). If an

intervention can increase RMR this negative energy balance will be met through the greatest contributor of metabolism.

Restricting energy intake through dieting is one method to achieve a negative energy balance. In early caloric restriction research, despite achieving a negative energy balance and modest body-weight reduction, caloric restriction interventions have also resulted in subsequent declines in RMR of as much as 30% (Lennon, Nagle, Stratman, Shrago, & Dennis, 1985; Mole, 1990). This reduction in RMR during caloric restriction-only interventions may occur to help a person maintain a set point for body weight (Woo, Daniels-Kush, & Horton, 1985; Sims, 1989; Keeseey, 1989; Wilmore, Stanforth, Hudspeth, et al., 1998).

Additionally, caloric restriction interventions have not demonstrated long-term weight loss (Broeder, Burrus, Svanevik, & Wilmore, 1992b; Wu, Gao, Chen, & van Dam, 2009). In studies that have mixed caloric restriction and exercise interventions, the groups that participated in aerobic exercise in addition to caloric restriction saw no significant change in RMR compared to declines in RMR in the caloric-restricted only groups (Lennon et al., 1985; Tremblay et al., 1986; Treuth, Hunter, & Williams, 1996). This suggests that aerobic exercise might have some positive effect on RMR in individuals that are calorically restricted.

Exercise training only interventions, both aerobic and resistance training, have also been proposed as potential methods to chronically increase RMR. Resistance exercise training has been recommended to raise RMR by increasing

fat-free body mass, specific to the relationship between fat-free mass and RMR (Broeder et al., 1992b). Broeder et al. (1992b) recruited 64 male subjects aged 18 – 35 years and randomly assigned them to one of three groups; control group (C, n = 20), a resistance-trained group (RT, n = 22), and an endurance-trained group (ET, n = 22). The training intervention groups completed prescribed training programs for 12 weeks. The RT group was designed to increase strength and fat-free mass utilizing a combination of exercises of free weights and Nautilus machines four- days- per- week for about 60 minutes per session (Broeder et al., 1992a). The ET group's intervention was a walk and/or jog program four days per week at intensities ranging from 70% to 85% $\dot{V}O_{2max}$ for 40-50 minutes per session (Broeder et al., 1992a). A telemetric heart-rate monitor measured accuracy of training session intensity for the ET group. The control group was instructed to maintain activity and dietary habits over the 12-week intervention period. All subjects were subjected to body composition analysis, resting metabolic rate analysis before and after the training intervention. The ET group was the only group to see a significant change in $\dot{V}O_{2max}$ after the training intervention. No groups saw a significant change in total body weight, however both the ET and RT groups showed significant decreases in relative body fat, fat weight, and waist-hip ratio. The RT group was the only group that saw a significant increase in fat-free weight after the intervention. Resting metabolic rate did not change in any of the groups after the 12-week training intervention, however the ET group did have a significant increase in average

resting RER (0.78 ± 0.01 vs. 0.83 ± 0.01 , pre-treatment vs. post-treatment, $p < 0.05$) The results from this study concur with other studies related to RMR changes after periods of either resistance or aerobic training (Binghman, Goldberg, Coward, Prentice, & Cummings, 1989; Tremblay et al., 1990).

However, Dolezal and Potteiger (1998) found that basal metabolic rate (BMR) was increased after a ten-week intervention of either resistance training only or concurrent resistance and endurance training. In this study 30 active men (20.1 ± 1.6 yrs) were assigned to one of three groups: endurance-training (ET, $n = 10$), resistance-training (RT, $n = 10$), and a combined endurance- and resistance-training group (CT, $n = 10$). The training interventions in this study were conducted over ten-weeks and consisted of exercising three days per week. The ET group participated in jogging and/or running exercise that ranged in intensity from 65% of age-derived maximum heart rate (HR_{max}) to 75-85% HR_{max} for 25-40 minutes per session. The RT group completed resistance training that included a combination Olympic style free-weight lifting and Universal machines that focused on all major muscle groups. The CT group completed all the training completed by both the ET and RT groups with both the endurance and resistance training performed on the same days. In this study, the ET group was the only group that had a significant decrease in total body mass (74.0 ± 5.2 kg vs. 71.5 ± 5.0 kg pre- vs. post-training, $p < 0.05$) and fat mass (8.8 ± 2.7 kg vs. 6.8 ± 1.6 kg, $p < 0.05$). All groups had significant decreases in body fat percentage ($p < 0.05$). Absolute BMR (kJ/day) significantly changed in all groups, with the RT and CT

groups showing significant increases in BMR (RT: $7,613.3 \pm 968.7$ kJ/day, $8,090.8 \pm 951.2$ kJ/day; CT: $7,454.9 \pm 964.2$ kJ/day, $7,801.8 \pm 980.6$ kJ/day, pre-post-training respectively, $p < 0.05$) whereas the ET group had a significant decline in BMR ($7,231.2 \pm 554.1$ kJ/day vs. $7,029.7 \pm 666.4$ kJ/day, $p < 0.05$). Additionally, when all 30 subjects in the study were analyzed collectively there was a significant correlation between the changes in pre- to post-training values for fat-free mass (FFM) and BMR ($r = 0.74$, $p < 0.01$). These findings concur with other reports that FFM is an intrinsic determinant of BMR (Bogardus et al., 1986; Weinsier, Schutz, & Bracco, 1992; Sjodin et al., 1996).

Both of these studies focused their research around the hypothesis specific to the relationship of increasing an individual's fat-free mass through resistance training to RMR increases, in recognition of the significant relation between fat-free mass and RMR (Broeder et al., 1992b; Jequier & Schutz, 1988). As can be seen from the results of these studies, the effectiveness of increasing fat-free mass and RMR through resistance training is mixed.

In addition to resistance training exercise interventions, aerobic or endurance training has been proposed to elevate RMR. Researchers have hypothesized that aerobic/endurance training will result in sustained increases in RMR. This includes research that has concluded that endurance trained athletes have higher resting metabolic rates than do age-matched non-trained athletes (Poehlman, Melby, & Badylak, 1991; Tremblay et al., 1986; Poehlman, Melby, Badylak, & Calles, 1989; Sjodin et al., 1996). Tremblay et al. (1986) directly

looked at their observations of endurance athletes having higher RMR than non-athletes. They recruited eight moderately obese women to complete an 11-week training program including five hours of aerobic exercise per week performed at a mean intensity of about 50% of $\dot{V}O_{2max}$. Among these individuals there was a significant increase in RMR, 8% higher than pre-training value in kcal/kg fat-free mass/min ($p < 0.01$) (Tremblay et al., 1986). A longitudinal study found increases in RMR with aerobic type training (Poehlman & Danforth, 1991). Nineteen older men and women (64.0 ± 1.6 yrs old) completed eight weeks of cycling training at an individualized intensity and duration to expend a given number of calories. In these subjects, there was no change in body composition, but there was a 10% increase in RMR after training ($p < 0.01$) which was associated with a higher rate of norepinephrine (NE) ($r = 0.57$; $p = 0.05$) (Poehlman & Danforth, 1991). This association of increased RMR with NE is one mechanism that has been suggested for RMR's increases in response to exercise interventions. Other mechanisms that have been suggested are; alterations in thyroid hormone concentrations [i.e. thyroxine (T_4) and triiodothyronine (T_3)], protein synthesis, glycogen resynthesis, and/or increased muscle mass or fat-free mass (FFM) (Lee et al., 2009; Poehlman, 1989; Wilmore et al., 1998).

Despite these findings in both cross-sectional and longitudinal studies, there are several other studies that have found contrasting results. In cross-sectional research there has been findings that current aerobic fitness level, training status, and fat-free mass are independent of resting metabolic rate in

men and women of a variety of ages (Poehlman, Melby, & Badylak, 1991; Broeder, Burrhus, Svanevik, & Wilmore, 1992a; Schulz, Nyomba, Alger, Anderson, & Ravussin 1991; Bosselaers, Buemann, Victor, & Astrup, 1994). Broeder et al., 1992b) observed that no change in RMR (presented as either absolute (kJ/min) or relative to total body weight (kJ/kg TWT/hr) or fat-free weight (kJ/kg FFW/hr)) occurred after a 12-week resistance or endurance training intervention. Wilmore et al. (1998) looked specifically at the effects of endurance training over 20 weeks of the HERITAGE Family Study on resting metabolic rate. In this large and heterogenetic sample RMR remained unchanged while there were small but significant changes in post-training relative body fat (-1.0%, $p<0.05$), fat mass (-0.6 kg, $p<0.05$) and fat-free mass (0.7kg, $p<0.05$) (Wilmore et al., 1998). Similar results were observed in a project that sought to not only examine the effect of a 12-week endurance exercise training program on RMR, but also provide insight into the possible mechanisms responsible for alterations in RMR that may occur (Lee et al., 2009). In this longitudinal study, Lee and colleagues observed no change in total body weight and RMR (both absolute and relative to total body weight and fat-free weight), yet they did observe a significant reduction in percent body fat and fat weight ($p<0.001$) in subjects that participated aerobic jogging and/or running three days per week (Lee et al., 2009). There was also no significant change in epinephrine (EPI), norepinephrine (NE), and total thyroxine (TT_4), but there was a significant decrease in free thyroxine (FT_4) ($p<0.05$).

There are discrepancies in the results when evaluating the affect of exercise training on resting metabolic rate. There are many possible explanations for these, but a few are; most of the subjects are men and much of the research is regularly focused on obese or overweight individuals, resting metabolic tests assessments in some studies were taken less than 24 hours after last exercise bout, exercise interventions used a variety of modalities and were limited in volume, intensity, and accurate measurement of training intensities. Many of these investigations encourage further research into the effect of different exercise modalities and intensities and their relationship to long-term changes to resting metabolic rate and possible mechanisms.

2.3 Substrate utilization changes at rest.

With the differences found in the research results specific to exercise intervention effects on RMR, the investigations have shifted to other possible changes that can lead to the amount of variability in weight loss and body composition changes related to exercise interventions. Bouchard et al. (1990) reported body weight loss ranging from 3 to 12 kg amongst a group of men residing in an isolated experimental station in a controlled environment independent of an exercise-induced energy deficit of 4.2 MJ/d for 84 days. It can be concluded that non-behavioral metabolic factors are also likely contributors to individual responses to exercise-induced weight and fat loss. Of the metabolic factors that have been considered as contributors to individual responsiveness to exercise interventions substrate utilization, specifically increases in fat oxidation

during rest have been suggested as another possible cause for weight and fat mass losses in those that have responded to the exercise interventions.

In regards to fat oxidation, both fasting and postprandial fat oxidation has been shown to increase for at least 24 hours following an exercise session (Votruba, Atkinson, Hirvonen, & Schoeller, 2002, Burton, Malkova, Caslake, & Gill, 2008, and Hansen, Shriver, & Schoeller, 2005). Additionally, in cross-sectional research it has been observed that endurance trained athletes have greater fat oxidation at rest than do untrained individuals (Romijn, Klein, Coyle, Sidossis, & Wolfe, 1993). Furthermore, other studies have shown that individuals in a fasted state have higher rates of carbohydrate oxidation compared to fat oxidation as measured analyzing the RER value using indirect calorimetry. Additionally, individuals are more likely to have long-term weight gain and weight regain after weight loss, independent of resting metabolic rate (Hopkins, Jeukendrup, King, & Blundell, 2011; Zurlo et al., 1990; Seidell, Muller, Sorkin, & Andres, 1992; Marra Scalfi, Covino, Esposito-Del Puente, & Contaldo, 1998; and Contaldo & Pasanisia, 2004). In research specific to exercise-induced changes in resting RQ or RER, there is variability amongst individuals specific to fat oxidation increases (Burton et al. 2008 and Goodpaster, Katsiaras, & Kelly, 2003). Furthermore, it has been demonstrated that the magnitude of fat oxidation increases is a strong predictor of other exercise-induced metabolic changes, such as the magnitude of change to postprandial lipid metabolism (Burton et al. 2008) and insulin sensitivity (Goodpaster et al. 2003). Barwell et al. (2009)

conducted an exercise intervention study to investigate the relationship of substrate utilization at rest and endurance-type training. Within the group of 55 fasted premenopausal women, resting RER significantly decreased (-0.03 ± 0.06 , $p < 0.05$) over the 7-week intervention (Barwell et al.; 2009). These results were paired with a significant decrease in fat mass (-1.0 ± 1.5 kg, $p < 0.01$) and percent body fat (-1.0 ± 1.5 , $p < 0.01$). Additionally, it was also concluded that with a decrease in fat mass and percent body fat, there was also a decrease in resting metabolic rate. These findings agree with other studies in which exercise interventions lead to decreases in fat mass, despite not increasing overall resting metabolic rate as was predicted by the investigators (Dolezal & Potteiger, 1998; and Sjodin et al. 1996). Moreover, many of the studies cited earlier when looking at RMR response to exercise interventions also noted that despite seeing no change in RMR they did see changes in resting RER values though not significant ($p=0.05$) (Broeder et al., 1992; Sjodin et al., 1996; Lee et al., 2009; and Campbell, Wallman, & Green, 2010).

The variability of RER at rest among individuals within a homogeneous group of trained cyclist has been demonstrated. Goedecke et al. (2000) found values that ranged from 0.718 to 0.927. The variability within the group persisted under exercising conditions and 59% of the variance in RER could be accounted for using; muscle glycogen content, training volume, proportion of type I fibers, free fatty acid concentration, lactate concentration, and percent of dietary fat intake (Goedecke et al., 2000). They also analyzed average RER values over a

set period of time. With only 59% of the variance explained with measured variables, there are indicators that there are more factors that can explain variability within each individual.

2.4 Sex-differences in metabolism

The concept of sex-differences in exercise metabolism has not been universally accepted in the scientific literature. Despite the lack of consensus regarding differences in metabolism between females and males, there is evidence that females rely more on fat oxidation during submaximal exercise intensities compared to males (Jansson, 1986; Friedlander et al., 1988; Tarnoplsky, MacDougall, Atkinson, Tarnopolsky, & Sutton, 1990; Horton, Pagliassotti, Hobbs, & Hill, 1998). These differences in substrate utilization are indicated by lower RER values as well as decreased net glycogen utilization and lower blood lactate concentrations in females compared to males when both are exercising at similar relative intensities (Nygaard, 1986; Tarnoplsky et al., 1990). The magnitude of the difference observed in studies varies with some observing a small sex-difference while others greater differences between the contribution of fat and carbohydrate oxidation in males and females. These differences in substrate utilization between studies might be explained by the intensity of exercise used in the studies. Horton et al. (1998) used lower intensity exercise (40% $\text{VO}_{2\text{max}}$) and observed a smaller sex-difference in substrate utilization compared to Costill et al. (1979) that used higher exercise intensity (65% $\text{VO}_{2\text{max}}$). Additionally, sex-differences have been shown to disappear at higher

intensity exercise (Froberg & Pedersen, 1984) as well as when the individuals are highly trained (Costill, Fink, Gretchell, & Ivy, 1979; Friedman & Kinderman, 1989).

Current available researches indicate that sex-differences in exercise metabolism seem to be mediated primarily by sex hormones such as estrogen and progesterone. Animal studies have observed that estrogen and progesterone work together to decrease the utilization of stored glycogen (Kendrick, Steffen, Rumsey, & Goldberg, 1987) as well as increase the availability of free fatty acids (FFA) during exercise in rat subjects (Ellis, Lanza-Jacoby, Gow, & Kendrick, 1994). Human studies have reported similar responses to estrogen and progesterone at rest and during exercise as was observed in the animal studies. Research of women during the follicular and luteal phases of the menstrual cycle has indicated lower carbohydrate oxidation during the luteal phase (Hackney, 1990; Hackney, McCracken-Compton, & Ainsworth, 1994). The luteal phase of the menstrual cycle is when the production of estrogen and progesterone is higher, allowing for these findings to be consistent with other research indicating that estrogen attenuates the utilization of carbohydrates. Despite the differences in metabolism that occur during the different phases of the menstrual cycle of women, research has indicated only decreases in aerobic capacity (VO_{2max} luteal phase: 52.8 ± 0.8 ml/kg/min, VO_{2max} follicular phase: 53.7 ± 0.9 ml/kg/min, $p=0.04$) and not anthropomorphic and physiological measurements (i.e. weight, percent body fat, hemoglobin concentration, hematocrit, maximum heart rate,

maximum minute ventilation, and maximum RER) and anaerobic and strength performance (Lebrun, McKenzie, Prior, & Taunton, 1995). Even with the observed decreased carbohydrate oxidation in women, especially during the luteal phase of the menstrual cycle, the exact mechanism of the sex hormones that lead to possible sex-difference in metabolism is still unknown and widely debated.

2.5 Non-linear Dynamics of Human Physiology

The recognition that the complexity of human anatomy and physiology is greater than the apparent rhythmic patterns easily observed has been presented by researchers implementing non-linear approaches to analyzing the fractal nature of the human body (Peng, Mietus, Liu, Hausdorff, Stanley, & Lipsitz, 2002; Goldberger & West, 1987). The use of non-linear approaches have lead to not only the observation that there is variability within physiological signals, but also important clinical information that could indicate disease states as well as physical and physiological adaptations within the human body. (Ferrario, Signorini, Magenes, & Cerutti, 2006; Seely & Macklem, 2004). Along the way, several non-linear analysis techniques have developed as well as a call for expanded investigation of the variability within many physiological signals (e.g. heart rate, respiratory rate, electroencephalography, metabolism, etc.) (Pincus, Galdstone, & Ehrenkranz, 1991; Voss, Schulz, Schroeder, Baumert, & Carminal, 2009; West, 2010). This increased popularity in variability analysis has lead to a considerable development of novel techniques by authors to distill the complexity

of time-series physiological data into measurements or graphical representations that uncover these underlying complexities (Bravi, Longtin, & Seely, 2011).

The science of variability analysis relies on a few common standards regarding the data being analyzed. These standards consist of data sampling, stationarity of data, lack of artifacts within the data, and measurement techniques (Seely & Macklem, 2004). Variability analyses is performed on a series of data sampled continuously or semi-continuously over a period of time where data can be converted to intervals between consecutive measurements (e.g. measured as R-R' intervals in heart rate, or inter-breath intervals in respiratory measurements) (Seely & Macklem, 2004). These series of sampled data should express the property stationarity, with means and standard deviations of the signal remaining the same throughout the period (Seely & Macklem, 2004). Additionally, data should lack artifact, with a minimal noise:signal ratio represented (Seely & Macklem, 2004). Both stationarity and the lack of artifact present limitations in characterizing variability, however these limitations can be addressed through measurement techniques. Various factors in measurement techniques such as body position, deep breathing, and sampling duration may influence the ability to complete variability analysis on data; therefore it is inappropriate to compare variability analysis from widely disparate measurement techniques and durations (Seely & Macklem, 2004).

2.6 Non-linear Variability Analysis Methods

Time and Frequency Domains

Time and frequency domain calculations represent the simplest method of identifying measures of variation over time using means, standard deviations, and graphic transformations of the frequency distribution within the data (box-plots, normal curves, etc.) (Seely & Macklem, 2004). Time domain analysis involving the overall standard deviation of heart rate signals (SDNN) to assess heart rate variability (HRV) has had extensive clinical and training adaptation research (Voss, 2007). This research indicates that an overall diminished in HRV using these methods portends poorer prognosis and/or increased mortality risk in patients with a variety of cardiovascular diseases as well as indicates possible overtraining of athletes (Kleiger, Miller, Bigger, & Moss, 1992; Routledge, Campbell, McFetridge-Dudle, & Bacon, 2010). Physiological data collected over time series are often mathematically transformed to graphical representations of the sinusoidal oscillations of the data (Seely & Macklem, 2004). The mathematical transformation developed over two centuries ago by Jean-Baptiste-Joseph Fourier (short-term Fourier transformation, STFT) is the most commonly frequency domain analysis used despite other methods that exist (e.g. wavelet, Hilbert) (Seely & Macklem, 2004). When using frequency domain analysis, the amplitude of the sine and cosine waves displays the contributions of the biological signals that are responsible for each wave (Seely & Macklem, 2004). This result of converting data from time series to frequency analysis is

termed spectral analysis because it provides an evaluation of the power (amplitude) of the contributing frequencies to the underlying biological signal (Bravi et al., 2011).

Even though both time and frequency analysis methods have strong clinical use, both have several limitations. First, stationarity of the data is required to derive a valid and meaningful analysis (Seely & Macklem, 2004). For example, when exercise intensity is varied, calculations of variability using these methods will not provide an accurate reflection of the actual variability of the signal (Voss et al., 2009). Additionally, a second potential confounding factor in characterizing variability with time and frequency domains, is that increases in baseline signal (e.g. heart rate, respiratory rate) may accompany diminished variability indices (Seely & Macklem, 2004). The clinical significance of this diminished variability as a result of increased baseline signal is unclear, because the prognostic significance of altered SDNN of HRV signals remains clinically useful (Seely & Macklem, 2004).

Poincaré Plots

The short-term and long-term dynamics of a biological signal can be graphically represented using Poincaré plots (Voss et al., 2009). This variability analysis is most commonly used in HRV studies providing a geometrical and nonlinear method of monitoring the dynamic changes of a biological signal. A Poincaré plot of HRV data is a diagram in which each R-R interval is plotted as a function of the previous R-R interval where the values of each pair of successive

R-R interval define a point in the plot (Hsu et al., 2012). Poincaré plots have been evaluated in a qualitative way using the visual pattern of the plot to categorize patients into functional classes indicating degrees of heart failure (Woo, Stevenson, Moser, Trelease, & Harper, 1992; Tulppo, Makikallio, Takala, Seppanen, Huikuri, 1996). Quantitative evaluation of these plots through the calculation of the short-term standard deviation (SD1) and the long-term standard deviation (SD2), as well as the ratio of these two signals (SD1/SD2) has been used to monitor the dynamic change of autonomic function during induction of general anesthesia (Kamen, Krum, & Tonkin, 1996; Woo et al., 2012). Additionally, Poincaré plots have been used in exercise studies looking at short and long-term HRV changes to assess the influence of exercise interventions on parasympathetic nervous system changes in sedentary individuals (Tulppo et al., 1996; Tulppo et al., 2003), endurance trained cyclists (Mourot, Bouhaddi, Perrey, Rouillon, & Regnard, 2004), and indications of overtraining (Mourot et al., 2004). The use of Poincaré plots has a relatively high intra-individual reproducibility and allows for effective assessment of changes within an individual, yet lacks validity in comparing inter-individual variability (Hsu et al., 2012).

Detrended Fluctuation Analysis

Detrended fluctuation analysis (DFA) is a variability analysis method developed specifically to distinguish between intrinsic fluctuations generated by complex systems and those caused by external or environmental stimuli acting on the system (Peng, Havlin, Stanley, & Goldberger, 1995). DFA involves the

removal of local trends, which are more likely related to external stimuli, in order to address the long-range correlations that are presumed to be due to intrinsic dynamics of the system (Seely & Macklem, 2004). The principle advantage to DFA over other non-linear techniques is the lack of confounding due to nonstationarity of the data (Seely & Macklem, 2004). This advantage has a trade-off of requiring a large amount of data points (greater than 8000) (Peng et al., 2002) lending the clinically useful applications of this non-linear analysis method to long duration sampling protocols; HRV in sleep apnea patients (Penzel, Kantelhardt, Grote, Peter, & Bundle, 2003), electroencephalography (EEG) changes in depressed males (Leistedt, Dumont, Lanquart, Jurystra, & Linkowski, 2007), and assessing depth of anesthesia using EEG readings (Jospin et al., 2007). Despite its advantages and the proven clinical significance of this technique, the usefulness of DFA method is still debated in the literature (Wilson & Francis, 2003).

2.7 Entropy Analysis

The non-linear variability analysis techniques that were originally developed allowed researchers to identify the physiological signals that had more complexity than their apparent rhythmic nature as well as quantify this complexity (Richman & Moorman, 2000; Costa, Goldberger, & Peng, 2002). These techniques have potentially important applications with respect to evaluating both dynamical models of biological control systems and bedside diagnostics (Costa et al., 20002). For example, some disease states and overtraining, appear to

degrade the variability of the physiological signal and reduce the adaptive capacity of the individual (Costa et al., 2002; Farrario et al., 2006). Therefore the decrease in variability has been proposed as a generic feature of pathologic dynamics (Richman & Moorman, 2000). However, certain pathologies, including cardiac arrhythmias like atrial fibrillation, may produce signs of increased variability as compared to healthy dynamics, despite the pathology and decreased adaptive capacity (Costa et al., 2002). This inconsistency may be a result of the fact that many of the traditional non-linear variability analysis techniques are based on single-scale analysis and do not take into account the complex temporal fluctuations inherent in healthy physiological control systems.

Entropy is a measure of disorder or randomness and entropy analysis techniques have been developed to characterize the system dynamics and to possibly provide a deeper understanding to the complexity of time series physiological signals that the traditional non-linear methods could not provide. Entropy analysis techniques are based in the idea that a state of a system at a certain instant is partially determined by its history and each new state carries a certain amount of new information (Costa et al., 2002). Random processes have high entropy because each new data point has as much information as the random point that preceded it (Richman, 2007). By calculating the randomness of a system as it moves through each of these individual states, entropy analysis techniques may provide further information about the variability of physiological systems.

Approximate Entropy

Approximate entropy (ApEn) was introduced in 1991 to measure the degree of irregularity or randomness within a series of data (Pincus et al., 1991). By using ApEn to analyze physiological time series, the complexity of the signal can be determined beyond what traditional non-linear analysis methods can provide. It was proposed that the method could be used to determine the complexity of an ever-adapting system such as physiological systems within the human body (Pincus et al., 1991). Pincus and Viscarello (1992) found low values of ApEn statistics in heart rate records of acidotic fetuses, and concluded that fetal distress led to greater order, or regularity of heart rate control.

Approximate entropy looks at a data series (of length N) and attempts to determine the pattern of the data within that series. Evaluating data sequences of length m and determining the likelihood that other runs of data in the data series of the same length m are similar within a specific tolerance (r) (Seely & Macklem, 2004). Thus, two parameters, m and r , must be fixed prior to calculating ApEn. The frequency of occurrence of repetitive runs and their prevalence is calculated to provide the ApEn value (Pincus & Goldberger, 1994). Small values of ApEn indicate greater regularity, which means that m and $m+1$ data series do not differ significantly from each other.

The main benefit of ApEn statistics use in physiology research is that it may be calculated for relatively short series of data (Seely & Macklem, 2004). Data series of 100-900 data points can be used to produce reliable and

reproducible results and some indications that ApEn is applicable to any system with as few as 50 data points (Pincus & Goldberger, 1994; Pincus, 2001). In contrast to time domain traditional non-linear variability analysis methods, ApEn requires analysis of consecutive data points, thus the order of the data is integral to the calculation of ApEn and must be preserved during data collection (Seely & Macklem, 2004). Additionally, significant noise within the data can compromise meaningful interpretation of ApEn calculations (Pincus & Goldberger, 1994).

With its early development and advantage of requiring a small number of data points, ApEn was the first entropy measure used in a wide range of clinical applications (Seely & Macklem, 2004). Initial clinical applications came when the use of ApEn was applied to detect changes in the regularity of infant's heart rate (Pincus, Cummins, & Haddad, 1993). These applications have expanded to include: heart rate variability in premature infants (Lake, Richman, Griffin, & Moorman, 2002), EEG changes in Alzheimer's patients (Adá solo et al., 2005), and respiratory changes in panic disorder patients (Caldirola, Bellodi, Caumo, Migliarese, & Perna, 2004). The application of ApEn statistics in these clinical situations seeks to give insight into the health of an individual and typically the more regular the signal is (lower ApEn calculation), the higher the change that there is some underlying dysfunction causing the regularity.

Sample Entropy

A modified algorithm to ApEn, sample entropy (SampEn), was proposed after almost a decade of research and clinical application of the entropy analysis

technique. Modifications were made to the ApEn statistic to address a few shortcomings that had been identified (Richman & Moorman, 2000). SampEn was proposed to alleviate the concern that ApEn is heavily dependent on the length of the recorded data (Costa et al., 2002). For smaller data sets the ApEn statistic had been shown to be lower than what it actually should have been (Richman & Moorman, 2000). Additionally, ApEn lacked consistency when compared to different data sets (Richman & Moorman, 2000). It appears that both of these shortcomings of ApEn are a result of inherent bias with how the ApEn statistic is calculated based on including self-matches (Seely & Macklem, 2004). The SampEn statistic changed how the entropy score was calculated to exclude self-matches resulting in a more robust entropy score (Richman & Moorman, 2000). These modifications also lead to SampEn having the advantage of not being effected by missing data points in a series of data as well as the ability to calculate an entropy score from relatively small data sets (as low as 100 data points), without the introduction of bias (Lake et al., 2002; Seely & Macklem, 2004). Many physiological processes can only generate a small amount of data points in a reasonable time frame, so having a non-linear analysis method requiring a small amount of data points is beneficial to researchers and allows for greater potential application of SampEn in the research of more physiological processes.

Similar to ApEn, SampEn has been used in a wide variety of clinical studies and applications. The first study to utilize SampEn found that the

SampEn statistic fell before the clinical signs of neonatal sepsis present themselves (Lake et al., 2002). The research using SampEn has continue to expand to include: identification of fetal distress (Ferrario et al., 2006), heart rate variability in obstructive sleep apnea patients (Al-Angari, & Sahakian, 2007), analysis of human postural sway (Ramdani, Seigle, Lagarde, Bouchara, & Bernard, 2009), and respiratory exchange ratio (RER) in trained individuals (Brown, 2013).

2.8 Non-linear variability analysis of RER

Time series data for a several physiologic variables exhibit moment to moment variability. Analyzing these variables as simple means and standard deviation does not allow for potentially useful information to be gained from this data (Biltz et al., 2011). Increases in fine scale variability trends to characterize healthy, adaptable physiology (West, 2006). A non-linear method that has been used to analyze time series data is Sample Entropy (SampEn). SampEn characterizes the inherent regularity of a data sequence and a higher entropy score implies decreased predictability of sequential values (Richman and Moorman, 2000; Biltz et al., 2011).

Non-linear variability analysis of breath-to-breath metabolic gas data has previously indicated that there is a measurable variability in the metabolic signal of inhaled O₂ and exhaled CO₂ in individuals in a resting state (Cadena, et al. 2007). Additionally, Cadena, Rodreguez, Medel, Infante, and Escalante (2008) demonstrated, under different experimental conditions, that this observed

metabolic variability is not the result of noise in the signal, but susceptible to be correlated with a physiological origin of metabolic variability. The SampEn variability analysis technique has been used to analyze RER data for signs of metabolic variability in a limited number of studies. Biltz, Harmon, Dengel, Unnitham, and Witten (2009) used SampEn to compare RER variability of lean and obese children and found that there was a trend for obese children to have a lower RER SampEn score compared to the lean children (lean: 1.72 ± 0.13 vs. obese: 1.49 ± 0.08 , $p=0.06$) during an exercise cycling test. Even though test results were not statistically significant, the trend indicates that the obese children oxidized substrates with less variability than did the lean children, and may support the researcher's hypothesis that obese children would demonstrate lower metabolic variability than their non-obese counterparts. Biltz et al. (2011) followed this research with another cross-sectional study in which trained female adolescent soccer players were compared to untrained controls. In this research it was observed that the trained soccer players had significantly higher RER SampEn scores (0.914 ± 0.433 vs. 0.5464 ± 0.139 , $p=0.026$) than compared to the untrained subjects during a 6-minute cycle protocol at 80% of their predetermined ventilator threshold (Biltz et al., 2011).

Recently, it was identified that a 16-week endurance training intervention increases an individual's RER SampEn score. In a group of novice college-aged marathon runners, RER SampEn scores significantly increased after a 16-week

training program when running at 65% of current predicted $\dot{V}O_{2\max}$ running pace (pre: 0.52 ± 0.34 , post: 0.71 ± 0.41 , $p < 0.01$) (Brown, 2013).

2.9 Conclusion

Resting metabolic rate accounts for 60-70% of total daily energy expenditure and the role of exercise in long-term changes in RMR is not completely understood. Exercise is regularly included as part of interventions for individuals whom are obese and/or have metabolic syndrome. Gaining an understanding of how exercise affects metabolism could be useful in suggesting appropriate volume and intensities for individuals. Similarly, understanding how specific modes of exercise influence overall metabolism can help athletes develop nutrition plans that match the adaptations they anticipate gaining from the intervention. As technology and analysis tools have become more available, the result is more accurate assessments of metabolic changes by means of non-invasive measurement tools. Traditional analysis of means and standard deviations provide one useful tool for monitoring metabolic adaptations. However, as variability analysis methods have become more widespread and easier to use, there might be deeper understanding of underlying physiological changes that can be learned. Research investigating the use of non-linear variability analysis on heart rate variability (HRV) has led the way in this field of research. There has been minimal research investigating the use of non-linear methods on metabolism, specifically respiratory exchange ratio (RER). Utilizing this analysis technique to assess breath-to-breath RER at rest and during exercise has the

potential to benefit both clinical and sport performance and the overall complexity of human metabolism.

Chapter 3: Methods

Chapter 3: Methods

The aim of this study was to determine the affect of marathon training on resting metabolic variables; resting metabolic rate (RMR), average respiratory exchange ratio (RER) and RER variability, as measured by SampEn, in healthy college-aged novice marathon runners. A secondary aim was to determine if breath-to-breath RER SampEn changes during steady state exercise and resting state correlate with changes in body weight, body fat (%BF and fat free mass (kg), as well as physiological variables; $\dot{V}O_{2max}$, ventilator threshold, and RER SampEn score collected during a graded exercise test following 16 weeks of marathon training.

3.1 Subjects:

Fifty-eight novice runners of the 106 enrolled (40 females: age = 22.76 ± 9.8 yrs, height = 1.67 ± 0.07 m, weight = 63.7 ± 7.78 kg; 18 males: age = 21.08 ± 0.93 yrs, height = 1.80 ± 0.06 m, weight = 74.51 ± 8.41 kg), in a marathon-training course in the Physical Activity Program at the University of Minnesota, volunteered to participate in the graded exercise testing part of this study. Seventeen of these participants also volunteered to participate in the additional resting metabolic rate testing (12 females: age = 20.34 ± 1.38 yrs, height = 1.67 ± 0.05 m, weight = 64.41 ± 8.27 kg, BMI = 23.0 ± 2.0 ; 5 males: age = 21.42 ± 1.46 yrs, height = 1.79 ± 0.05 m, weight = 74.29 ± 7.45 kg, BMI = 23.2 ± 2.9). The subjects were healthy college-aged students who had no known metabolic disorders and had not trained for a marathon in the last twelve months. All the

subjects completed the marathon training and ran the Eau Claire Marathon on May 4, 2014. The purpose and risks of participation in the marathon training and the study were carefully explained to each subject before giving written consent to participate. The experimental protocol was approved by the University of Minnesota Institutional Review Board.

3.2 Experimental Procedures:

All participants completed two sets of testing sessions, one before and one after a 16-wk marathon training program. Pre-training testing (PRE) was performed in early December prior to the start of the preplanned training program, and post-training testing (POST) was performed in late April, within 2-wks of scheduled marathon. Each testing session consisted of two visits (one to an indoor track and one to a performance laboratory) to complete the physiological performance measurements. The first visit was to the university's field house to complete a two-mile time trial (2-mile TT) with a group of fellow runners on a 200-meter indoor track. During the 2-mile TT subjects were instructed to give their best effort for their current training status and were provided with volunteer counters to keep track of laps and finish time. Times were taken with hand timers and runners were given verbal encouragement and informed of their split time at the one-mile mark. At a later date within the two weeks of this time-trial, subjects reported to the Human and Sports Performance Laboratory (HSPL) in the School of Kinesiology at the University of Minnesota for

the second visit. Measurements of height and weight, body composition, and maximal oxygen consumption ($\dot{V}O_{2\max}$) were measured during the second visit.

For the second visit, subjects reported to the HSPL in a post-absorptive state (≥ 3 hrs post prandial). Body weight (BW) and height were measured using a electric scale with an attached stadiometer (ProDoc: Detecto Scale, Webb City, Missouri). Body density (D_b) was determined by hydrostatic weighing performed in a standalone water tank using a chair suspended from four force transducers (EXERTECH, La Crescent, Minnesota). Ten weighing trials were performed, and the average of the three weights that were within 0.1 kg was used in the equation. Residual volume (RV) was estimated using the equation developed by Goldman and Becklace (1959). Percentage of body fat (%BF) was determined from D_b using the Siri equation (Siri, 1961). Fat mass (FM) was calculated from BF and BW, and fat-free mass (FFM) was recorded as the difference between BW and FM.

Following body composition assessment, subjects performed a continuous, graded, multistage treadmill exercise test to determine $\dot{V}O_{2\max}$. The protocol for the test was developed on the basis of the protocol used in Popp (2009) and included changes in both speed and grade. The test began with a warm-up at 3.1 mph and 0% grad for one-minute followed by a six-minute stage at 0% incline and a speed that was 65% of PRE-2-mile TT pace. This extended stage was intended to provide enough data points so that metabolic variability analysis could be completed with data gathered during this stage. Following

stages were one-minute in length with speed and/or grade increasing each stage until subject reach volitional maximal effort. Criteria for reaching maximal effort was achievement of an age predicted maximal heart rate ($220 - \text{age}$), $\text{RER} > 1.1$, or reaching >16 on a Borg rate of perceived exertion scale.

Participants that volunteered to complete the RMR measurement were required to return to the HSPL for a third visit. This visit was scheduled within 7-days of the previous visit. Subjects were instructed to transport themselves to the HSPL by motor vehicle at their assigned time between 6:00 and 9:00 a.m. after a restful night's sleep (≥ 8 hrs), in a fasted state (≥ 12 -hr post prandial), refraining from caffeine, tobacco, or alcohol consumption in past 12hours, as well as refraining from vigorous physical activity for 36hours. The time period of 36hours after last exercise session has been shown to eliminate the residual effects of the last exercise bout on RMR (Poehlman et al., 1989). Additionally, as RMR is effected by the menstrual cycle (Donahoo, Levine, & Melanson, 2004), all females were asked which day of their menstrual cycle they were on during pre-testing so that post-testing could be schedule during the same menstrual phase (Campbell et al., 2010). After BW and height were measured, subjects were fitted with a telemetric HR monitor (Polar, Kempele, Finland) and facemask with a pneumotachometer (MGC diagnostics, St. Paul, Minnesota). A 10-minute period of quiet rest in a supine position then preceded the ensuing RMR measurement. For the RMR measurement, $\dot{V}\text{O}_2$, RER, and HR were monitored for 35-min. Ambient room temperature was maintained at $\sim 21 \pm 1^\circ\text{C}$, lights dimmed, and

noise was kept at a minimum during testing. Subjects were instructed to remain awake but as quiet as possible without distractions from external devices before and throughout the entire RMR session. Breath-to-breath (btb) $\dot{V}O_2$ and RER values measured during the last 25-min of the 35-min measurement period were averaged and used to calculate RMR using the Weir equation (kilocalories (kcal) = $[(1.1 \times \text{RER}) + 3.9] \times \dot{V}O_2$) (Weir, 1949). Additionally, RER values were for non-linear sample entropy (SampEn) variability analysis. HR was measured continuously throughout the entire testing period using a Polar s810 wrist computer (Polar, Kempele, Finland) with R-R measurements used in heart rate variability (HRV) calculations.

All metabolic data collected during $\dot{V}O_{2\max}$ and RMR measurements were collected using a facemask and pneumotachometer metabolic measurement system (MGC diagnostics, St. Paul, MN). The instrument's gas sensors were calibrated immediately before each test with two different gas mixtures (21% O_2 /79% N_2 and 5 % CO_2 /12% O_2 /83% N_2) in addition to air-flow rate and volume calibration using a three-liter syringe (MGC diagnostics, St. Paul, MN). Collection and analysis of all metabolic cart data was completed using Breezesuite software version 6.4.1 (MGC diagnostics, St. Paul, MN).

3.3 Non-Linear Data Analysis:

The raw data collected during both the graded exercise testing and the RMR measurement was checked for artifact prior to analysis. Sample entropy (SampEn) scores were calculated using Kubios Heart Rate Variability software

Version 2 (University of Kuopio, Kuopio, Finland). Kubios was originally designed as a heart rate variability program, but its use has been validated for variability analysis of other biological time-series data, such as RER (Tarvainen, Niskanen, Lipponen, Ranta-aho, & Karjalainen, 2009). Default values of $m = 2$ and $r = 0.2 \times SD$ were used during the SampEn analysis. SampEn was chosen based on the lack of bias in the measure when compared to other entropy scores and due to limited number of data points.

3.4 Statistical Analysis:

All variables were checked for normality prior to statistical analysis. Pre- and post-RER variability (measured by SampEn), average RER, $\dot{V}O_{2max}$, body weight, fat free mass, and percent body fat were analyzed using a paired sample t-test. Correlations between physiologic variables and performance variables were assessed using linear regression. There was no difference between males and females in regards to the variables measured, so they were considered together as one group. Results are presented as means \pm standard deviations. An α level of <0.05 was considered to be statistically significant. Statistical analysis was completed using IBM SPSS Statistics 24.0.0 software (IBM, Armonk, New York).

Chapter 4: Resting metabolic rate is not affected by marathon training in novice college-aged runners

Chapter 4: Resting metabolic rate is not affected by marathon-training in novice college-aged runners (for submission to the International Journal of Sport Nutrition and Exercise Metabolism)

Resting metabolic rate is not affected by marathon-training in novice college-aged runners.

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Abstract:

RHODES, G.S.; LUNDSTROM, C.J.; and INGRAHAM, S.J. Resting metabolic rate is not affected by marathon-training in novice college-aged runners.

Purpose: To assess the effects of an endurance exercise training intervention on resting metabolic rate (RMR) in college-aged, healthy, novice marathon runners. **Methods:** Seventeen novice runners (12 female, 5 male, ages 18-23), enrolled in a marathon-training course at the University of Minnesota, volunteered to participate in the study. Participants completed 16-weeks of an organized marathon training along with pre- and post-training performance, physiology, and RMR testing. Lab testing included 2-mile time trial, body composition by hydrostatic underwater weighing, graded exercise test on a treadmill, and 35-minute RMR test. Repeated-measures paired t-test was used to compare average RMR measures pre- and post-training and bivariate correlations were used to explore the relationship between RMR and changes in body weight (BW) and fat-free mass (FFM). **Results:** RMR, expressed either in absolute terms (kcal/day) or relative to FFM (kcal/kg FFM), did not change from pre- to post-training (1438.02 ± 274.61 kcal/day PRE, 1491.71 ± 260.37 kcal/day POST, $p=0.92$; 27.52 ± 6.27 kcal/FFM*day⁻¹ PRE, 27.49 ± 5.23 kcal/FFM *day⁻¹ POST, $p=0.98$). Despite the lack of significant changes in RMR or BW for the group, large variability of individual changes indicated a significant positive correlation between changes in RMR and changes in FFM ($r=0.647$, $p=0.005$). **Conclusion:** Following a controlled 16-week marathon training program, RMR did not increase for novice, college-aged, runners. Individually, changes in RMR demonstrated a positive relationship associated with increases in FFM, suggesting that exercise that develops lean muscle mass might play a larger role in increase daily total energy expenditure.

Introduction

Obesity, metabolic disorders, and the comorbidities associated with them, such as hypertension, diabetes, hyperlipidemia, and hypercholesterolemia, are an epidemic issue worldwide (Campbell, Wallman, & Green, 2010). Overeating alone does not explain the dramatic increase in the prevalence in obesity and metabolic disorders, and the Surgeon General suggests that significant health benefits can be obtained by including a moderate amount of physical activity on most days of the week (U.S. Department of Health and Human Services, 1996). The effect exercise has on metabolism has been looked at extensively to identify the role it plays in establishing a negative energy balance and possibly correcting this epidemic in obesity and metabolic disorders.

Resting metabolic rate (RMR) is the minimum energy required for maintenance of critical body functions. Small but chronic changes in the total daily energy expenditure and specifically the RMR, which comprises approximately 60-70% of the total daily energy expenditure in humans, have a cumulative impact on the maintenance of body mass (Poehlman, Melby, & Bradylak, 1988). Therefore, any intervention or lifestyle change that chronically alters RMR may have important implications for energy balance and possible metabolic disorders.

The role in which exercise influences RMR has been looked at with mixed results. Cross-sectional studies have reported RMR to be higher in individuals with higher fitness status or training levels (Gilliat-Wimberly, Manore, Woolf,

Swan, & Carroll, 2001; Sullo, Cardinale, Brizzi, Fabri, & Maffulli, 2004). Where as interventional studies have mixed results regarding the influence exercise has on RMR. Some researchers have reported increases in RMR ranging from 3% to 7.7% with either endurance or resistance training (Trembley, Fontaine, Poehlman, Mitchell, Perron, & Bouchard, 1986; Lemmer et al., 2001; Morio et al., 1998), while others have seen no change in RMR following similar exercise interventions (Poehlman & Danforth, 1991; Wilmore et al., 1998; Lee, Sedlock, Flynn, & Kamimori, 2009; Campbell et al., 2010). The recognition of the discrepancies has lead some researchers to suggest that there might be an intensity-duration threshold needed for exercise to produce a prolonged effect on RMR (Molé, 1990).

The purpose of this study was to further examine the effects of an endurance exercise training intervention on RMR. The recruitment of healthy, novice participants provides the opportunity to investigate how a longitudinal high-volume training program influences RMR when completion of a marathon, and not weight reduction was the focus of the intervention.

Methods

Seventeen novice runners (12 females: age = 20.34 ± 1.38 yrs; 5 males: age = 21.42 ± 1.46 yrs), enrolled in a marathon-training course in the Physical Activity Program at the University of Minnesota, volunteered to participate in this study. The subjects were healthy college-aged students who had no known metabolic disorders and had not trained for a marathon before. All the subjects

completed sixteen weeks of programmed marathon-training and successfully finished the same marathon. The purpose and possible risks of participation in the training program and the study were carefully explained to each subject before giving consent to participate. The experimental protocol was approved by the University of Minnesota Institutional Review Board.

Pre- and post-training body composition, aerobic capacity, and resting metabolic rate (RMR) were assessed for all subjects. Pre-testing started two-weeks prior to the initiation of the standardized marathon-training protocol, and post-testing took place during the final week of the 16-wk training program prior to the marathon. Subjects reported to the Human and Sports Performance Laboratory (HSPL) at the University of Minnesota for all testing sessions.

One week prior to both pre- and post-training testing periods, subjects participated in a two-mile time trial (2-mile TT) as part of the Physical Activity class. Subjects reported to the university's field house to complete the 2-mile TT with a group of fellow runners on a 200-meter indoor track. During the 2-mile TT, subjects were instructed to give their best effort for their current training status and were provided with volunteer counters to keep track of laps completed and provide verbal encouragement. Times were taken with hand timers and runners were informed of their split time at one-mile.

Body composition and a graded exercise test were both completed during the first visit to the HSPL. Subjects were required to arrive in a fasted state (≥ 3 hrs post prandial) and without consuming any caffeine, tobacco, or alcohol in

the twelve-hours prior to testing. Body weight (BW) and height were measured using an electric scale with an attached stadiometer (ProDoc: Detecto Scale, Webb City, Missouri). Body density (D_b) was determined by hydrostatic weighing performed in a standalone water tank using a chair suspended from four force transducers (EXERTECH, La Crescent, Minnesota). Ten weighing trials were performed, and the average of three weights within 0.1 kg were used to calculate D_b . Residual volume (RV) was estimated using the equation developed by Goldman and Becklace (1959). Percentage of body fat (%BF) was determined from D_b using the Siri equation (Siri, 1961). Fat mass (FM) was calculated from BF and BW, and fat-free mass (FFM) was recorded as the difference between BW and FM.

$\dot{V}O_{2\max}$ was measured using a graded exercise test performed on a treadmill (Woodway Pro, Waukesha, WI), and breath-to-breath (btb) gas samples were collected using a metabolic cart (MGC diagnostics Ultima CPX, St. Paul, MN). Gas sensors were calibrated prior to data collection using two different gas mixtures, 21% O_2 /79% N_2 and 5 % CO_2 /12% O_2 /83% N_2 , (MGC diagnostics, St. Paul, MN). Air-flow rate and volume were calibrated using a three-liter syringe (MGC diagnostics, St. Paul, MN). Subjects were fitted with a facemask and pneumotachometer (MGC diagnostics, St. Paul, MN). Heart rate was monitored continuously throughout the testing using a heart rate monitor strap and Polar s810 wrist computer (Polar, Kempele, Finland). The graded exercise test protocol used in this study was developed and validated by previous researchers (Popp,

2009). The protocol included a one-minute warm-up session at 3.1 mph followed by a six-minute stage at a speed that corresponded to 65% of their 2-mile TT pace. This extended stage was intended to provide enough data points so that metabolic variability analysis could be completed. The following stages were one-minute long and increased in speed or grade until each subject reached volitional maximal effort. Criteria for reaching maximum effort was achievement of an age predicted maximal heart rate ($220 - \text{age}$), $\text{RER} > 1.1$, or reaching a rate of perceived exertion ($\text{RPE} > 16$ on the Borg scale (Borg, 1986). $\dot{V}\text{O}_{2\text{max}}$ was considered the highest $\dot{V}\text{O}_2$ recorded during the test for successive minute intervals. The same testing protocols were administered during post-testing.

RMR testing for each subject was completed within seven-days of their body composition and aerobic capacity test. All RMR measurements were completed 36hrs after the last exercise session; this time period has been shown to eliminate the residual effects of the last exercise bout on RMR (Poehlman et al., 1989). Additionally, as RMR is affected by the menstrual cycle (Donahoo, Levine, & Melanson, 2004), all females were asked which day of their menstrual cycle they were in during pre-testing so that post-testing could be scheduled during the same menstrual phase (Campbell et al., 2010). Subjects reported to the HSPL for their testing after a full nights rest ($\geq 8\text{hrs}$) and in a fasted state ($\geq 12\text{hrs}$ post prandial). All resting tests were completed between 6:00 and 9:00a.m. to control for diurnal metabolism variations, and pre- and post-testing for each subject were completed at the same time of day. After BW and height were

measured, subjects were fitted with a HR monitor strap (Polar, Kempele, Finland) and facemask with a pneumotachometer (MGC diagnostics, St. Paul, Minnesota). A ten minute period of quiet rest in a supine position then preceded the ensuing RMR measurement. For the RMR measurement, $\dot{V}O_2$, RER, and HR were monitored for 35 minutes. Ambient room temperature was maintained at $\sim 21 \pm 1^\circ\text{C}$, lights dimmed, and noise was kept at a minimum during testing. Subjects were instructed to remain awake but as quiet as possible without distractions from external devices before and throughout the entire RMR session. Breath-to-breath $\dot{V}O_2$ and RER values measured during the last 25-min of the 35-min measurement period were averaged and used to calculate RMR using the Weir equation (kilocalories (kcal) = $[(1.1 \times \text{RER}) + 3.9] \times \dot{V}O_2$) (Weir, 1949). Collection and analysis of all metabolic cart data was completed using Breezesuite software version 6.4.1 (MGC diagnostics, St. Paul, MN). Raw data was checked for artifact prior to analysis.

Data were analyzed using the SPSS Statistics version 24 (IBM, Armonk, NY). All variables were checked for normality prior to statistical analysis. There was no difference between male and females in regard to the variables measured, so they were analyzed together as one group. All data are reported as mean \pm standard deviations (SD). A repeated-measures paired *t*-test analysis was used to examine the effects of the 16-wk marathon-training program on all dependent variables. The relationship between changes in RMR and changes in weight (BW and FFM) was analyzed using a bivariate correlation analysis, and

the Pearson's r was calculated. Statistical significance was accepted for all tests at $P < 0.05$.

Results

The subjects' time to complete a two-mile time trial decreased (15.47 ± 2.62 min PRE, 14.24 ± 2.24 min POST, $p < 0.001$) while their $\dot{V}O_{2\max}$ increased as well (51.79 ± 9.06 ml/kg*min⁻¹ PRE, 55.06 ± 7.50 ml/kg*min⁻¹ POST, $p < 0.001$) after the 16-weeks of training for the marathon. Body weight and percent body fat did not change during the training period (67.31 ± 9.08 kg PRE, 67.72 ± 8.79 kg POST, $p = 0.56$; 18.56 ± 5.95 PRE, 18.96 ± 6.08 POST, $p = 0.64$ respectively).

	Pre-testing		Post-testing		p-value
	mean \pm SD	Range	mean \pm SD	Range	
Age (yrs)	20.65 ± 1.45	18.4 – 23.2			
Height (m)	1.71 ± 0.07	1.6 – 1.9	1.71 ± 0.07	1.6 – 1.9	0.548
Weight (kg)	67.31 ± 9.08	51.4 – 87.2	67.72 ± 8.79	53.1 – 83.6	0.558
BMI (kg/m ²)	23.07 ± 2.23	19.7 – 27.8	23.23 ± 1.97	20.6 – 26.8	0.542
% Body fat	18.56 ± 5.95	7.5 – 25.7	18.96 ± 6.08	7.0 – 27.8	0.638
Fat-free mass (kg)	54.91 ± 9.02	41.5 – 71.1	55.01 ± 9.19	40.8 – 69.2	0.832
Fat mass (kg)	12.40 ± 4.28	5.5 – 18.6	12.71 ± 4.29	5.2 – 21.0	0.661
$\dot{V}O_{2\max}$ (ml/kg*min ⁻¹)	51.19 ± 8.71	40.0 – 70.5	55.06 ± 7.50	43.3 – 69.1	<0.001
2-mile time trial (minutes)	15.47 ± 2.62	11.3 – 20.2	14.24 ± 2.24	10.5 – 17.1	<0.001

Table 1: Pre- and post-training values for measured physiologic and performance variables in marathon-training subjects.

Both absolute and relative RMR did not change following the 16-weeks of training to run a marathon (1438.02 ± 274.61 kcal/day PRE, 1491.71 ± 260.37 kcal/day POST, $p=0.92$; 27.52 ± 6.27 kcal/FFM*day⁻¹ PRE, 27.49 ± 5.23 kcal/FFM *day⁻¹ POST, $p=0.98$). The correlation between the change in RMR (kcal/day) and BW of the subjects was not significant ($r=0.326$, $p=0.202$). However, there was a significant positive correlation between changes in RMR (kcal/day) and FFM ($r=0.647$, $p=0.005$).

	Pre-testing		Post-testing		p-value
	mean \pm SD	Range	mean \pm SD	Range	
RMR (ml O₂/min)	206.78 \pm 38.71	147.6 – 276.7	206.26 \pm 37.54	143.8 – 206.3	0.965
RMR (kcal/day)	1438.02 \pm 274.61	1048.1 – 1988.8	1491.71 \pm 260.37	1099.8 – 1857.9	0.915
RMR (kcal/kg FFM/day)	27.52 \pm 6.27	16.03 – 42.53	27.49 \pm 5.23	18.49 – 42.40	0.981

Table 2: Resting metabolic rate pre- and post-training 16-week marathon-training program.

Discussion

The aim of this study was to observe the effect of 16-weeks of controlled marathon training on RMR. The results of this study concurred with other studies; when increasing physical activity through an organized training program was the primary focus of an intervention, RMR, expressed either in absolute terms (kcal/day) or relative terms (kcal/FFM*day⁻¹), remained unchanged from pre- to post-training (Bingham, Goldberg, Coward, Prentice, & Cummings, 1989; Tremblay, Nadeau, Després, St-Jean, Thériault, & Bouchard, 1990; Broeder et al., 1992b). In a study by Bingham et al. (1989), basal metabolic rate (BMR) did

not change in six normal-weight subjects that completed 9-weeks of jogging and isometric exercise training. Similarly, Broeder et al. (1992b) did not see changes in both absolute or relative RMR in volunteers completing 12-weeks of either resistance-training, endurance run training, or a control group. Another longitudinal study, Tremblay et al. (1990) had subjects using cycle-ergometer training at about 50% $\dot{V}O_{2max}$ for 14-weeks and did not see changes in BMR. Despite the wide variety of training stimuli and intensity, the results from all these studies, including ours, agree that long-term changes in RMR are not seen when a training program is provided to individuals.

Regardless, similar conclusions between these results and those described above, there are conflicting results from other studies (Tremblay et al., 1986; Lennon, Nagle, Stratman, Shrager, & Dennis, 1984). In these studies, RMR was seen to significantly increase following endurance training programs which lasted >11-weeks. In addition to seeing increases in RMR these studies also reported significant decreases in subjects BM and FM following their training interventions (Tremblay et al., 1986; Lennon et al., 1984). These decreases in BM and FM observed in these studies are also in conflict with the results from this study as well as those from other research mentioned (Bingham et al., 1989; Tremblay et al., 1990; Broeder et al., 1992b).

The discrepancies among longitudinal studies investigating the effect of exercise on RMR is unclear. Explanations for these inconsistencies have included 1) the methods used to present RMR data as absolute values or

normalized for relative values to body weight or composition, 2) timing of the RMR measurement in relationship to the last bout of exercise, 3) genetic-related factors, and 4) variations in mode of exercise and the starting fitness level of subjects.

In this study, there was no significant difference in RMR between pre- and post-training regardless of the method used to express RMR as absolute values or normalized for body weight or composition. Therefore, the method used to express RMR data cannot account for the differences observed in the present study and those previously reported. Interestingly, despite no significant change in body weight and/or body composition in this study, a wide range of change in BW and FFM was observed amongst the participants. These changes ranged from 5.84 kg gain to 4.67 kg loss in total body weight as well as 3.42 kg loss to 3.44 kg gain in fat-free mass. The changes in FFM pre- to post-training significantly correlated with changes in RMR in kcal/day ($r=0.65$, $p=0.005$) (Figure 1). This positive correlation between increases in FFM and RMR supports other studies that have seen similar correlation despite which training methods are used (Broeder et al., 1992a; Poehlman et al., 1986; Lee et al., 2009). These results suggest that increasing FFM may play a large role in altering RMR, and therefore justifies further research into what causes these interactions.

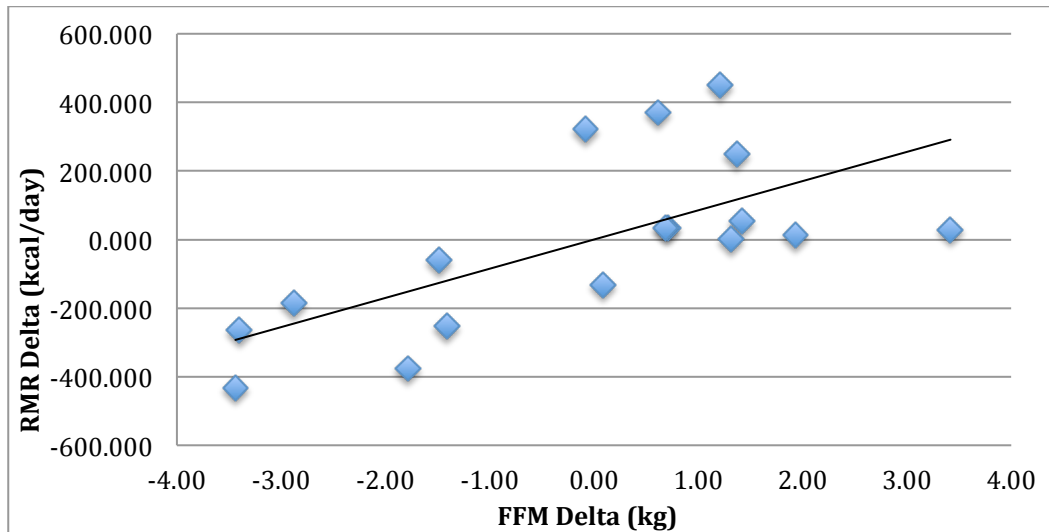


Figure 1: Correlation between changes in RMR (kcal/day) and FFM (kg) pre- to post-training ($r=0.65$, $p=0.005$)

Another possible reason for discrepancies among studies may be related to the timing of the RMR measurement after the last bout of exercise. RMR measurements in this study were done at least 36hours after any strenuous physical activity. It has been shown that RMR is significantly higher than the pre-training RMR when measured less than 18hours after physical activity (Broeder et al., 1992a). Yet, if a minimum of 24hours of recovery is given after strenuous exercise, RMR measurements return to levels similar to pre-training values (Sjödín, Forslund, Weterterp, Andersson, Forslund, & Hambreus, 1995). If time since previous physical activity was not controlled for before the RMR measurement during both the pre- and post-training, conclusions regarding the long-term effect exercise has on RMR might not be reliable. Thus, in controlling for previous physical activity in this study, the long-term effects of endurance training on RMR can be better assessed.

The large range of RMR measurements and individual differences in changes in BW, FFM, and RMR indicate that there might be genetic influence on metabolism and the role exercise has on altering long-term metabolic changes. It has been suggested by Bouchard et al. (1989) using data from the HERITAGE family study that there are responders and non-responders to any given adaptation we expect to see from endurance training stimulus. These inheritable traits of RMR and responses to short-term training interventions have been observed by others as well (Bogardus, et al., 1986; Poehlman et al., 1986). This suggests that when analyzing the effect that endurance exercise has on RMR the need to take into account individual differences and the possibility of genetic influences is critical. As a result of the sample population including over 50% women, the influence of sex may have skewed the overall results of the relationship between training, FFM and RER.

Finally, the discrepancies in the results may come from variations in the exercise intervention in the study as well as the starting fitness level of the participants. Previous studies that have observed significant increases in RMR have had participants whom are overweight and/or lower fitness levels at the start of the intervention (Lennon et al., 1984; Tremblay et al., 1986). The current study's participants had pre-training BMI and $\dot{V}O_{2max}$ values that indicated they were of normal weight and above average aerobic fitness (23.07 ± 2.23 and 51.79 ± 9.06 ml/kg * min⁻¹ respectively). These pre-training characteristics are similar to other studies that did not find RMR change following a longitudinal

exercise intervention despite varied modes of exercise intervention (Broeder et al., 1992b; Lee et al., 2009; Campbell et al., 2010).

In conclusion, there are no significant changes in RMR measured in absolute terms ($\text{mL O}_2 / \text{min}$ or kcal/day) or relative to FFM following 16-weeks of endurance training developed to prepare novice athletes to run a marathon. In addition, despite no significant change in BW or FFM, there was a significant correlation between changes in RMR and FFM. These results support other research in that endurance exercise alone does not increase RMR, but that there is a correlation between RMR and FFM. These results indicate an increase in FFM might be the key aspect in increasing RMR when using an organized training intervention to alter RMR with the ultimate goal of obtaining a negative energy balance.

Chapter 5: Marathon training increases fat oxidation at rest and during submaximal exercise, but does not change respiratory exchange ratio variability

Chapter 5: Marathon training increases fat oxidation at rest and during submaximal exercise, but does not change respiratory exchange ratio variability (for submission to the Journal of the International Society of Sports Nutrition)

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Abstract:

RHODES, G.S.; LUNDSTROM, C.J.; and INGRAHAM, S.J. (2017) Marathon training increases fat oxidation at rest and during submaximal exercise, but does not change respiratory exchange ratio variability. **Purpose:** To assess the effect of a 16-week marathon-training program on respiratory exchange ratio (RER) and breath-to-breath (btb) RER, measured by sample entropy (SampEn) at rest and during submaximal steady-state running, in college-aged, novice marathon runners. **Methods:** Seventeen novice runners (12 female, 5 male, ages 18-23), enrolled in a marathon-training course at the University of Minnesota, volunteered to participate in the study. Participants completed 16-weeks of an organized marathon training along with pre- and post-training performance, physiology, and RMR testing. Lab testing included 2-mile time trial, body composition by hydrostatic underwater weighing, graded exercise test on a treadmill, and 35-minute RMR test. Repeated-measures paired t-test was used to compare average RER (avg RER) and RER SampEn measures pre- and post-training for both resting and steady-state submaximal running. Additionally, bivariate correlation was used to explore the relationship between Avg RER and RER SampEn with and changes in body weight (BW), fat mass (FM), percentage body fat (%BF), $\dot{V}O_{2max}$, 2-mile time trial performance, and resting metabolic rate (RMR). **Results:** Avg RER significantly decreased pre- to post-training at rest (PRE, 0.92 ± 0.03 ; POST, 0.81 ± 0.06 ; $p < 0.001$) and during steady-state submaximal running (PRE, 0.95 ± 0.05 ; POST, 0.86 ± 0.05 ; $p < 0.001$). However, RER SampEn did not change with training (Rest: -0.001 ± 0.37 , $p = 0.99$; Running, -0.054 ± 0.36 , $p = 0.54$). **Conclusion:** Participants increased fat oxidation at rest and during steady-state submaximal running, but no change in RER SampEn. The effects of endurance exercise on increasing fat oxidation was not associated with changes in BW, FM, %BF, or $\dot{V}O_{2max}$ as well as changes in the variability of substrate utilization. This suggests that endurance exercise can alter overall substrate utilization, but there are other interactions needed to alter varied components of obesity and metabolic syndrome.

Introduction

It has been established that the metabolic response to exercise training varies between individuals. (Barwell, Malkova, Leggate, & Gill, 2009; Snyder, Donnelly, Jabobsen, Hertner, & Jakicic, 1997). Compliance to exercise interventions has been identified as a contributing factor to these differences (King et al., 2007); though when this is accounted for, differences in weight, fat mass, and metabolic response between participants are observed (Barwell et al., 2009; Snyder et al., 1997). It has been suggested that individual differences in compensatory adjustments to dietary intake and spontaneous activity to the increased exercise energy expenditure are responsible for this variability (King et al., 2007). The decrease in energy expended in spontaneous activity has been shown to lead to smaller than expected changes in total energy expenditure and in substrate utilization in response to exercise interventions (Goran & Poehlman, 1992; Levine, Eberhardt, & Jensen, 1999). This suggests that individual differences in the weight loss and the metabolic response to endurance exercise interventions could be influenced by behavioral differences of the participants.

However, these behavior differences cannot explain all the variations in response to exercise interventions. For example, research has shown variations in resting metabolic rate (RMR), body weight (BW), and percent body fat (%BF) in participants of studies with highly controlled environments and dietary intake (Bouchard et al., 1990; Poehlman & Danforth, 1991; Wilmore et al., 1998; Lee,

Sedlock, Flynn, & Kamimori, 2009). In these studies, it is unlikely that all the variations in response to the interventions could be explained by differences in compensatory behavioral changes between subjects. Therefore, non-behavioral metabolic factors are likely to play an additionally role in individual responsiveness to exercise-interventions. Changes in substrate utilization could provide further insight into variations in response to exercise interventions (Goodpaster, Wolfe, & Kelley, 2002; Corpeleijn, Saris, & Blaak, 2009).

Improvements in the technology of indirect calorimetry breath-to-breath (btb) gas detection methods have increased the insight into the effect of exercise on metabolism (Goedecke, Gibson, Grobler, Collins, Noakes, & Lambert, 2000; Romijn, Klein, Coyle, Sidossis, & Wolfe, 1993). The analysis of respiratory exchange ratio (RER) has been validated to assess changes in substrate utilization in response to exercise (Bergman & Brooks, 1999, Romijn et al., 1993) as well as training status and performance predictions (Ramos-Jiménez et al., 2008; Bellar & Judge, 2012). Indeed, studies have reported lower RER values (indicating high fat and low carbohydrate oxidation) at rest in endurance-trained vs untrained individuals (Romijn et al., 1993), as well as high variations in resting RER between individuals in a homogeneous group of highly trained cyclists (range: 0.72 to 0.93) (Goedecke et al., 2000). Additionally, exercise-induced increase in resting fat oxidation in obese individuals has been demonstrated to predict other exercise-induced metabolic changes (Goodpaster, Katsiaras, &

Kelley, 2003; Blaak & Saris, 2002). The effect of exercise on resting RER may contribute to individual variations in the response to an exercise intervention.

Traditional calculations of mean and standard deviation (SD) of RER over a given period of time of rest have provided limited and mixed results regarding substrate utilization at rest in response to exercise (Broeder, Burrhus, Svanevik, & Wilmore, 1992a; Lee et al., 2009; Campbell et al., 2010). Traditional linear methods (mean and standard deviation) of analyzing physiological data does not take into account the potentially valuable information that is found in the fine scale variability of time series data. Therefore, exploration into non-linear analysis methods may provide additional insight into RER changes in response to exercise training.

Non-linear analysis of time-series data has become common in clinical and exercise physiology (Bravi, Longtin, & Seely, 2011). Heart rate variability (HRV) research has provided a model for non-linear analysis techniques to quantify and correlate variability produced by a physiological signal. Variability analysis allows a quantifiable value to be assigned to a dynamic physiological process through mathematical reconstruction into a signal variable (Voss et al., 2009). Studies have shown that increases in HRV following training interventions are indications of healthy adaptations to this stimulus (Tulppo et al., 2003). Likewise, it predicts that a similar increase in variability of substrate utilization may also occur.

There are multiple non-linear techniques to assess variability, yet many of these are limited in use for physiological variables due to the large time series data sets needed (Seely & Macklem, 2004). To overcome that limitation, the non-linear analysis technique of sample entropy (SampEn) was developed to analyze data with as few as 50 data points (Voss et al., 2009). SampEn seeks to quantify the inherent irregularity of a system or the sequential pattern of data generation of a system (Richman & Moorman, 2000). This is accomplished through calculating a conditional probability that a set of points of length m will agree with the next set of points $m+1$ within a given tolerance of r (usually $0.2 \times SD$). A higher SampEn score implies decreased predictability of sequential values.

Understanding the inherent variability in substrate utilization may provide valuable information about the effect exercise has on resting metabolism from both a clinical and sports performance perspective. This is the first known published study that analyzes the changes in RER SampEn during rest after an endurance training intervention. Breath-to-breath (btb) gas analysis variability has previously been shown not to be just artifact, but provide insight into underlying metabolism status in a clinical setting (Biltz, Harmon, Dengel, Unnithan, & Witten, 2009; Cadena Méndez et al., 2008). Additionally, RER SampEn scores during steady-state exercise were positively correlated with training status in adolescent female athletes (Blitz, Unnithan, Brown, Marwood, Roche, & Holloway, 2011), as well as shown to increase after endurance training in novice college-aged runners (Brown, 2013). The purpose of this study was to observe

the effect of a 16-week marathon-training program on RER variability during a resting state, as measured by SampEn, in healthy college-aged novice marathon runners.

Methods

Seventeen novice runners (12 females: age = 20.34 ± 1.38 yrs; 5 males: age = 21.42 ± 1.46 yrs) enrolled in a marathon-training course in the Physical Activity Program at the University of Minnesota and volunteered to participate in this study. The subjects were healthy college-aged students who had no known metabolic disorders and had not trained for a marathon in the last twelve months. All the subjects completed sixteen weeks of programmed marathon-training and successfully finished the same marathon. The purpose and possible risks of participation in the training program and the study were carefully explained to each subject before giving consent to participate. The experimental protocol was approved by the University of Minnesota Institutional Review Board.

Pre- and post-training body composition, aerobic capacity, and resting metabolic rate (RMR) were assessed for all subjects. Pre-testing started two-weeks prior to the initiation of the standardized marathon-training protocol, and post-testing took place during the final week of the 16-week training program prior to the marathon. Subjects reported to the Human and Sports Performance Laboratory (HSPL) at the University of Minnesota for all testing sessions.

One week prior to both pre- and post-training testing periods, subjects participated in a two-mile time trial (2-mile TT) as part of the Physical Activity

class. Subjects reported to the university's field house to complete the 2-mile TT with a group of fellow runners on a 200-meter indoor track. During the 2-mile TT subjects were instructed to give their best effort for their current training status and were provided with volunteer counters to keep track of laps completed and provide verbal encouragement. Times were taken with hand timers and runners were informed of their split time at one-mile.

Body composition and a graded exercise test were both completed during the first visit to the HSPL. Subjects were required to arrive in a fasted state (≥ 3 hrs post prandial) and without consuming any caffeine, tobacco, or alcohol in the twelve-hours prior to testing. Body weight (BW) and height were measured using an electric scale with an attached stadiometer (ProDoc: Detecto Scale, Webb City, Missouri). Body density (D_b) was determined by hydrostatic weighing performed in a standalone water tank using a chair suspended from four force transducers (EXERTECH, La Crescent, Minnesota). Ten weighing trials were performed, and the average of three weights within 0.1 kg were used in the equation. Residual volume (RV) was estimated using the equation developed by Goldman and Becklace (1959). Percentage of body fat (%BF) was determined from D_b using the Siri equation (Siri, 1961). Fat mass (FM) was calculated from BF and BW, and fat-free mass (FFM) was recorded as the difference between BW and FM.

$\dot{V}O_{2\max}$ was measured using a graded exercise testing performed on a treadmill (Woodway Pro, Waukesha, WI), and btb gas samples were collected

using a metabolic cart MGC diagnostics Ultima CPX, St. Paul, MN). Gas sensors were calibrated prior to data collection using two different gas mixtures, 21% O₂/79% N₂ and 5 % CO₂/12% O₂/83% N₂, (MGC diagnostics, St. Paul, MN). Air-flow rate and volume were calibrated using a three-liter syringe (MGC diagnostics, St. Paul, MN). Subjects were fitted with a facemask and pneumotachometer (MGC diagnostics, St. Paul, MN). Heart rate was monitored continuously throughout the testing using a heart rate monitor strap and Polar s810 wrist computer (Polar, Kempele, Finland). The graded exercise test protocol used in this study was developed and validated by previous researchers (Popp, 2009). The protocol included a one-minute warm-up session at 3.1 mph followed by a six-minute stage at a speed that corresponded to 65% of their 2-mile TT pace. This extended stage was intended to provide enough data points so that metabolic variability analysis could be completed with the data gathered during this stage. The following stages were one-minute long and increased in speed or grade until each subject reached volitional maximal effort. Criteria for reaching maximum effort was achievement of an age predicted maximal heart rate (220-age), RER > 1.1, or reaching a rate of perceived exertion (RPE) > 16 on the Borg scale (Borg, 1982). $\dot{V}O_{2max}$ was considered the highest $\dot{V}O_2$ recorded during the test for successive minute intervals. The same testing protocols were administered during post-testing.

RMR testing for each subject was completed within seven-days of their body composition and aerobic capacity test. All RMR measurements were

completed 36hrs after the last exercise session; this time period has been shown to eliminate the residual effects of the last exercise bout on RMR (Poehlman et al., 1989). Additionally, as RMR is affected by the menstrual cycle (Donahoo, Levine, & Melanson, 2004), all females were asked which day of their menstrual cycle they were in during pre-testing so that post-testing could be scheduled during the same menstrual phase (Campbell et al., 2010). Subjects reported to the HSPL for their testing after a full night of rest (≥ 8 hrs) and in a fasted state (≥ 12 hrs post prandial). All resting tests were completed between 6:00 and 9:00am to control for diurnal metabolism variations, and pre- and post-testing for each subject were completed at the same time of day. After BW and height were measured, subjects were fitted with a HR monitor strap (Polar, Kempele, Finland) and facemask with a pneumotachometer (MGC diagnostics, St. Paul, Minnesota). A 10-minute period of quiet rest in a supine position then preceded the ensuing RMR measurement. For the RMR measurement, $\dot{V}O_2$, RER, and HR were monitored for 35-min. Ambient room temperature was maintained at $\sim 21 \pm 1^\circ\text{C}$, lights dimmed, and noise was kept at a minimum during testing. Subjects were instructed to remain awake but as quiet as possible without distractions from external devices before and throughout the entire RMR session. $\dot{V}O_2$ and RER values measured during the last 25-min of the 35-min measurement period were averaged and used to calculate RMR using the Weir equation (kilocalories (kcal) = $[(1.1 \times \text{RER}) + 3.9] \times \dot{V}O_2$) (Weir, 1949).

Collection and analysis of all metabolic cart data was completed using Breezesuite software version 6.4.1 (MGC diagnostics, St. Paul, MN). Raw data was checked for artifact prior to analysis. Kubios Heart Rate Variability software Version 2 (University of Kuopio, Kuopio, Finland) was used for non-linear analysis of btb RER data. Kubios was originally designed as a heart rate variability program but can also be used for other biological time series data such as RER (Tarvainen, Niskanen, Lipponen, Ranta-aho, & Karjalainen, 2009). Default values of $m = 2$ and $r = 0.2 \times SD$ were used during the SampEn analysis. SampEn analysis was chosen based on the lack of bias in the measure when compared to other entropy scores and due to the limited number of data points.

Data were analyzed using the SPSS Statistics version 24 (IBM, Armonk, NY). All variables were checked for normality prior to statistical analysis. There was no difference between male and females in regard to the variables measured, so they were considered together as one group. A repeated-measures paired *t*-test analysis was used to examine the effects of the 16-week marathon-training program on all dependent variables. Bivariate correlations were used to explore the relationships between RER and RER SampEn scores at rest and during submaximal exercise, and physiological and performance characteristics. All data are reported as mean \pm standard deviations (SD) and an α level of $P < 0.05$ was considered to be statistically significant.

Results

	Pre-testing		Change with marathon training	
	mean \pm SD	Range	mean \pm SD	Range
Age (yrs)	20.65 \pm 1.45	18.4 – 23.2		
Height (m)	1.71 \pm 0.07	1.6 – 1.9	0.00 \pm 0.00	-0.01 – 0.01
Weight (kg)	67.31 \pm 9.08	51.4 – 87.2	0.41 \pm 2.81	-4.67 – 5.85
BMI (kg/m ²)	23.07 \pm 2.23	19.7 – 27.8	0.16 \pm 1.07	-2.04 – 2.18
% Body fat	18.56 \pm 5.95	7.5 – 25.7	0.40 \pm 3.45	-3.89 – 7.88
Fat-free mass (kg)	54.91 \pm 9.02	41.5 – 71.1	0.10 \pm 2.00	-3.42 – 3.44
$\dot{V}O_{2max}$ (ml/kg*min ⁻¹)	51.19 \pm 8.71	40.0 – 70.5	3.87 \pm 3.64*	-1.40 – 12.20
2-mile time trial (minutes)	15.47 \pm 2.62	11.3 – 20.2	-1.23 \pm 0.98*	-3.10 – 0.05

Table 3: Baseline pre-training values for measured physiologic and performance variables. Amount of change after completing 16-week marathon training.

* p<0.01 for change with exercise training

Baseline characteristics and group changes in response to 16-weeks of marathon training are shown in Table 1. The group as a whole significantly increased $\dot{V}O_{2max}$ (7.6%, p<0.001) and decreased 2-mile TT time (8.0%, p<0.001). Body weight, percent body fat, and fat-free mass did not change significantly during the training period (0.41 \pm 2.81 kg, p= 0.56; 0.40 \pm 3.45 %, p= 0.64; and 0.10 \pm 2.00 kg, p= 0.83; respectively).

Average RER (avg RER) during steady-state submaximal running at the same pre- and post-training pace, maintaining equal relative work rate, significantly decreased from PRE to POST (0.95 \pm 0.05 vs. 0.86 \pm 0.05,

p<0.001). Similarly, avg RER during rest decreased (0.92 ± 0.03 vs. 0.81 ± 0.06 , p<0.001) following the exercise intervention. Both of these decreases in avg RER suggest that there was a shift towards higher fat oxidation following 16-weeks of marathon-training during exercise and at rest despite no significant change in RMR (1438.02 ± 274.61 vs. 1491.71 ± 260.37 , p=0.915). Across the group, there was a wide individual variation in degree of change in avg RER during exercise and rest. These changes ranged from +0.04 to -0.16 during submaximal running as well as from +0.06 to -0.21 during rest.

	Pre-testing		Post-testing		p-value
	mean \pm SD	Range	mean \pm SD	Range	
Submaximal Running					
avg RER	0.95 ± 0.05	0.86 – 1.08	0.86 ± 0.05	0.78 – 0.93	<0.001*
RER SampEn	0.90 ± 0.36	0.30 – 1.27	0.95 ± 0.24	0.22 – 1.25	0.544
Resting					
avg RER	0.92 ± 0.03	0.86 – 0.97	0.81 ± 0.06	0.74 – 0.98	<0.001*
RER SampEn	0.79 ± 0.34	0.33 – 1.58	0.79 ± 0.28	0.28 – 1.28	0.991
RMR (kcal/day)	1438.02 ± 274.61	1048.1 – 1988.8	1491.71 ± 260.37	1099.8 – 1857.9	0.915

Table 4: Average RER and RER SampEn score during 6-min steady-state running at 65% of pre-testing 2-mile TT pace. Resting metabolic testing average RER, RER SampEn score, and RMR measurements pre- and post-training 16-week marathon-training program.

Non-linear analysis of RER variability showed no significant change during submaximal running or rest. RER SampEn scores during exercise changed from 0.90 ± 0.36 pre-training to 0.95 ± 0.24 post-training ($p=0.544$). Similarly, RER SampEn scores during rest changed from 0.79 ± 0.34 pre-training to 0.79 ± 0.28 post-training ($p=0.991$). Even though neither of these showed significance over the 16-week training intervention, it can be seen that during steady-state submaximal exercise RER measurements do have higher variability as measured by SampEn than during rest.

Bivariate analysis was used to describe the relationships between resting and submaximal running RER and different physiological variables; indicate no significant correlations were found (Table 3). Additionally, RER SampEn correlations to the same physiological variables indicate no significant correlation (Table 5).

Avg RER	Body Weight (kg)	Fat Mass (kg)	% Body Fat	$\dot{V}O_{2max}$ (ml/kg/min)	2-mile TT (min)	RMR	Avg RER resting	Avg RER during exercise
Resting	-0.246 (.34)	-0.229 (.38)	-0.235 (.36)	-0.034 (.90)	-0.386 (.13)	-0.215 (.40)	1.00	-0.20 (.94)
Submax. running	0.185 (.48)	0.030 (.91)	0.002 (.99)	-0.219 (.40)	0.239 (.36)	0.050 (.85)	-0.20 (.94)	1.00
RER SampEn								
Resting	-0.371 (.14)	-0.085 (.75)	-0.037 (.89)	0.340 (.18)	-0.110 (.67)	-0.076 (.77)	-0.186 (.48)	-0.202 (.44)
Submax. running	-0.54 (.84)	-0.087 (.74)	-0.093 (.72)	0.473 (.06)	0.072 (.78)	0.157 (.55)	0.112 (.67)	-0.323 (.21)

Table 5: Correlation matrix for avg RER and RER SampEn at rest and during steady-state running. N= 17; values are correlation coefficients, with p-values in parentheses. Statistical significances was set at $p < 0.05$.

Discussion

The primary purpose of this research was to observe the effect of a 16-week marathon-training program on metabolic variability measured using the non-linear analysis method of SampEn. RER SampEn did not significantly change from pre- to post-training at either rest or during submaximal running in this study ($p=0.991$ and 0.544 , respectively). These results are in contrast to previous results found in our lab where RER SampEn increased during submaximal running in a similar group of novice runners training for a marathon the previous year (Brown, 2013). However, similar to Brown (2013), there is no correlation between change in RER SampEn during exercise and change in avg RER, $\dot{V}O_{2max}$, 2-mile TT, or %BF. The lack of correlation of RER SampEn and performance in a 2-mile time trial is not surprising in either of these studies.

Running a 2-mile TT at the highest intensity possible is both too short a duration and too high of intensity to allow substrate utilization to influence performance outcome (Brown, 2013).

The discrepancy between no change in RER SampEn in this study and an increase in RER SampEn in Brown (2013) might be explained by research that has identified the large influence of heterogeneity of muscle fiber type (Goedecke et al., 2000) and metabolism phenotypes (Assfalg et al., 2008) on substrate utilization. In contrast to Brown (2013), this study included measurements of RER SampEn at rest, but again did not show significant change following the training intervention. The initial hypothesis that endurance training would increase RER SampEn was based on cross-sectional data indicating that trained individuals had higher RER SampEn than age and gender match untrained individuals. Even though this study did not confirm this hypothesis, there still might be a connection with exercise training and substrate utilization changes. Conversely, dietary tendencies might play a more prominent role. Future research that strictly controls diet intake and includes a control group may allow for further insight into correlations between RER SampEn and substrate utilization.

Even with no observed significant change in RER SampEn, avg RER at rest and during submaximal running decreased from pre- to post-training, indicating an increase in fat oxidation at rest and during exercise. The avg RER decrease during submaximal running was from 0.95 to 0.86, which corresponds to an increase in the relative rate of fat oxidation from 17% to 48%. Similarly, the

decrease in avg RER at rest indicates an increase in relative rate of fat oxidation from 27% to 65%. These changes in avg RER at rest and during exercise are consistent with other studies that used a variety of exercise intervention types (Barwell et al., 2009; Snyder et al., 1997). Conversely, these changes in avg RER were not associated with change in RMR or changes in FM. The lack of significant change in both RMR and avg RER is in agreement with other studies that use similar endurance exercise interventions (Barwell et al., 2009; King et al., 2007; Marra, Scalfi, Covino, Esposito-Del Puente, & Contaldo, 1998).

While the significant change in avg RER and lack of significant change in RER SampEn provide insight into substrate utilizations, the variation of change between individuals is lost in these analyses. A more in-depth look at the raw data indicates large individual differences in response to the exercise intervention. Similar variation between individuals has been previously demonstrated when comparing trained and untrained subjects at rest and during low to moderate-intensity steady-state exercise (Goedecke et al., 2000; Helge et al., 1999). This variability in response to exercise interventions could be explained by the concept of responders and non-responders to exercise that has been suggested by the HERITAGE family study (Bouchard et al., 1999).

In conclusion, this study observed the change in avg RER at rest and during submaximal exercise after a 16-week marathon-training program. This change represents a shift in metabolic substrate utilization towards higher levels of fat oxidation during rest and exercise. However, the shift towards increased fat

oxidation was not associated with change in body weight or fat mass in the participants. Additionally, non-linear analysis of btb RER did not show significant change following the exercise intervention. The lack of agreement between traditional (means \pm SD) and non-linear variability analysis of RER (SampEn) supports the idea of individual difference in response to exercise. The study questions whether there is value in continuing to use non-linear variability analysis of RER to further understand substrate utilization adaptations to exercise. The addition on a control group as well as strict control over subject's dietary intake over the entire intervention period might provide more in-depth understanding regarding the effective methods in altering metabolic variables in humans. A larger sample population with equal representation of males and females may also show the sex interaction specific to these findings. However, the results may have important implications for metabolic research in regard to the effect of endurance running on substrate utilization.

Chapter 6: Heart rate variability increases during submaximal exercise yet does not change at rest in novice marathon runners.

Chapter 6: Heart rate variability increases during submaximal exercise yet does not change at rest in novice marathon runners. (for submission to the Journal of the Strength and Conditioning Research)

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Abstract:

RHODES, G.S.; LUNDSTROM, C.J.; and INGRAHAM, S.J. (2017) Heart rate variability increases during submaximal exercise yet does not change at rest in novice marathon runners. **Purpose:** To assess the effect of a 16-week marathon-training program on heart rate variability (HRV) measurements at rest as well as during steady-state submaximal running using time domain, frequency domain, and entropy measurements. **Methods:** Twelve novice college-aged runners (9 females, 3 males; age: 20.78 ± 1.38) enrolled in a marathon-training course, volunteered to participate in the study. Participants completed pre- and post-training lab testing as well as 16-weeks of organized training as a group. Lab testing consisted of an indoor 2-mile time trial, body composition assessment, a graded exercise test to determine $\dot{V}O_{2max}$, and a 35-minute resting metabolic rate test. Data for HRV analysis was collected during a 6-minute steady-state run at an intensity of 65% of pre-testing 2-mile time trial pace, as well as during resting metabolic rate testing. All heart rate data was recorded using a Polar s810 wrist computer and HRV indices were calculated using Kubios software. Matched paired *t*-test was used to compare HRV variables pre- and post-training. **Results:** There was a significant decrease in heart rate (HR) (165 to 157 bpm, pre to post, $p=0.03$) and increase mean R-R interval length (366 to 386 ms pre to post, $p=0.01$) during steady-state submaximal exercise, indicating improved running economy. Additionally, the SD1 calculation from the Poincaré plot increased significantly. No time domain, frequency domain, or entropy measurements of HRV significantly changed at rest pre- to post- marathon-training. **Conclusion:** Time and frequency domain variability analysis indicated changes in HRV during steady-state submaximal exercise. These changes may indicate either an increase in the parasympathetic nervous system (PNS) and/or a decrease in the sympathetic nervous system (SNS). In contrast, no significant change in HRV indices at rest were observed, suggesting that adaptations to the PNS and SNS that affect HRV during exercise correspond to other physiological stimulus. Additionally, the use of non-linear entropy variability analysis did not significantly change in either resting or exercising situations, suggesting that it is possible that each variability analysis measurement is sensitive to varied physiological adaptations.

Introduction

Endurance exercise training interventions often focus on altering long-term physiological adaptations. Finding minimal invasive indicators of adaptation and training status in athletes has been of interest in exercise physiology and sports medicine. The most common variables observed relate to adaptive changes in the neuroendocrine system and specifically heart rate (HR). Heart rate, and specifically heart rate variability (HRV), has been shown to detect adaptations in the autonomic nervous system (ANS) gained from endurance exercise training (Pichot et al., 2002; Buchheit et al., 2008; Manzi et al., 2009).

HRV refers to the variations in the R-R intervals and is a useful signal for understanding the status of the ANS. Normal variability in the HR is due to autonomic neural regulation of the heart and the circulatory system (Saul, 1990). The balancing action of the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS) branches of the ANS controls HR. For example, a low SNS activity or a high PNS activity causes cardio-deceleration (Acharya, Joseph, Kannathal, Min Lim, & Suri, 2006). The degree of variability in the HR provides information about the function of the ANS on the HR and its ability to respond to stimuli. Negative adaptation to training, as in overtraining, is generally associated with reductions in HRV (Bosquet, Merkari, Arvisas, & Aubert, 2008). Conversely, increases in fitness (Lee, Wood, & Welsch, 2003; Vesterinen, Hakkinen, Hynynen, Mikkola, Hokka, & Nummela, 2013), and

exercise performance (Atlaoui, Pichot, Lascoste, Barale, Lacour, & Chatard, 2007) are thought to be associated with increases in HRV.

There are several different ways to analyze the R-R variability in HR, and to date most HRV research has focused on time, frequency, and entropy domain measurements (Bravi, Longtin, & Seely, 2011). A common way of looking at HRV has been the time domain analysis of calculating a mean and standard deviation of the R-R signal (SDNN) during a given period of time. Poincaré plots provide a visual representation of the HR data, and quantitative information can be gained when short-term (SD1) and long-term (SD2) variability is calculated. These values can be combined to calculate the cardiac sympathetic index ($SD2/SD1$) and cardiac vagal index ($\log_{10}(16 \times SD1 \times SD2)$) (Bravi et al., 2011). These variables have been successfully applied in the assessment of cardiac contractility (Patangay, Zhang, & Lewicke, 2009), the tracking of aging (Ahmad et al., 2009), and other clinical cardiac assessments (Bravi et al., 2011).

Entropy domains were developed to measure the regularity of the underlying processes generating the signal and to quantify the inherent regularity of the time series data generated by the system (Richman & Moorman, 2000; Bravi et al., 2011). Approximate entropy (ApEn) and sample entropy (SampEn) are such methods. They assess the regularity of the data through calculating a conditional probability that a set of points of length m will agree with the next set of points $m+1$ within a given tolerance of r (usually set at $0.2 \times SD$). As ApEn is the precursor of SampEn; the difference between ApEn and SampEn is that

SampEn excludes the comparison of sets of points with themselves avoiding the bias of self-matches, and thus SampEn is a preferred method of analysis (Bravi et al., 2011). When using SampEn, higher values imply lower predictability of sequential values. These higher SampEn values have been associated with healthy and adaptable physiology, whereas lower SampEn values may indicate a disease state or overtraining (Lake, Richman, Griffin, & Moorman, 2002; Manzi et al., 2009).

The use of HRV analysis is growing among professional and recreational athletes with new commercial devices available to consumers. Additionally, exercise scientist have been using HRV analysis to assess overtraining in elite endurance athletes (Earnest et al., 2004; Plews et al., 2012), predict performance (Manzi et al., 2009), and monitor training adaptations of sedentary and trained athletes (Tulppo et al., 2003; Buchheit et al., 2010). Despite the extensive number of studies and the high level of use amongst coaches and athletes, there is not a definitive understanding about how HRV responds to endurance training. For example, Buchheit et al. (2011) showed improvements in maximal aerobic running speed and 10km run time had moderate ($r=0.52$) and large ($r=-0.73$) correlations respectively, with increases in resting HRV. In contrast, other studies have observed decreases in HRV despite increases in fitness measurements, such as $\dot{V}O_{2\max}$ (Iellamo et al., 2002).

Most literature and commercial HRV assessment tools focus on using time and frequency domain measures and have not utilized entropy domains as

measurement tools. Additionally, there is a gap in the literature looking at the effect that organized marathon-training programs have on HRV in novice, recreational athletes. The purpose of the current study is to assess the effect of a 16-week marathon-training program on HRV measurements at rest as well as during steady-state submaximal running using time domain, frequency domain, and entropy measurements.

Methods

Twelve novice runners (9 females: age: 20.44 ± 1.26 yrs, weight: 66.97 ± 7.34 kg, BMI: 23.53 ± 1.93 , $\dot{V}O_{2\max}$: 45.35 ± 4.52 ml/kg/min; 3 males: age: 21.80 ± 1.43 yrs, weight: 76.04 ± 9.95 , BMI: 24.56 ± 3.00 , $\dot{V}O_{2\max}$: 57.80 ± 11.31 ml/kg/min) (Table 1) enrolled in a marathon-training course in the Physical Activity Program at the University of Minnesota and volunteered to participate in this study. The subjects were healthy college-aged students who had no known metabolic disorders and had not trained for a marathon in the last twelve months. All the subjects completed sixteen weeks of programmed marathon-training and successfully finished the same marathon. The purpose and possible risks of participation in the training program and the study were carefully explained to each subject before giving consent to participate. The experimental protocol was approved by the University of Minnesota Institutional Review Board.

Pre- and post-training body composition, aerobic capacity, and resting metabolic rate (RMR) were assessed for all subjects. Pre-testing started two-

weeks prior to the initiation of the standardized marathon-training protocol, and post-testing took place during the final week of the 16-week training program prior to the marathon. Subjects reported to the Human and Sports Performance Laboratory (HSPL) at the University of Minnesota for all testing sessions.

One week prior to both pre- and post-training testing periods, subjects participated in a two-mile time trial (2-mile TT) as part of the Physical Activity class. Subjects reported to the university's field house to complete the 2-mile TT with a group of fellow runners on a 200-meter indoor track. During the 2-mile TT, subjects were instructed to give their best effort for their current training status and were provided with volunteer counters to keep track of laps completed and provide verbal encouragement. Times were taken with hand timers, and runners were informed of their split time at one-mile.

Body composition and a graded exercise test were both completed during the first visit to the HSPL. Subjects were required to arrive in a fasted state (≥ 3 hrs post-prandial) and without consuming any caffeine, tobacco, or alcohol in the twelve-hours prior to testing. Body weight (BW) and height were measured using an electric scale with an attached stadiometer (ProDoc: Detecto Scale, Webb City, Missouri). Body density (D_b) was determined by hydrostatic weighing performed in a standalone water tank using a chair suspended from four force transducers (EXERTECH, La Crescent, Minnesota). Ten weighing trials were performed, and the averages of three weights within 0.1 kg were used to calculate D_b . Residual volume (RV) was estimated using the equation developed

by Goldman and Becklace (1959). Percentage of body fat (%BF) was determined from D_b using the Siri equation (Siri, 1961). Fat mass (FM) was calculated from BF and BW, and fat-free mass (FFM) was recorded as the difference between BW and FM.

$\dot{V}O_{2max}$ was measured using graded exercise testing performed on a treadmill (Woodway Pro, Waukesha, WI), and breath-to-breath gas samples were collected using a metabolic cart (MGC diagnostics Ultima CPX, St. Paul, MN). Gas sensors were calibrated prior to data collection using two different gas mixtures, 21% O_2 /79% N_2 and 5% CO_2 /12% O_2 /83% N_2 , (MGC diagnostics, St. Paul, MN). Air-flow rate and volume were calibrated using a three-liter syringe (MGC diagnostics, St. Paul, MN). Subjects were fitted with a facemask and pneumotachometer (MGC diagnostics, St. Paul, MN). Heart rate was monitored continuously throughout the testing using a heart rate monitor strap and Polar s810 wrist computer (Polar, Kempele, Finland). The graded exercise test protocol used in this study was developed and validated by previous researchers (Popp, 2009). The protocol included a one-minute warm-up session at 3.1 mph followed by a six-minute stage at a speed that corresponded to 65% of their pre-testing 2-mile TT pace. This extended stage was intended to provide enough data points so that metabolic and HR variability analysis could be completed with the data gathered during this stage. The following stages were one-minute long and increased in speed or grade until each subject reached volitional maximal effort. Criteria for reaching maximum effort was achievement of an age predicted

maximal heart rate ($220 - \text{age}$), $\text{RER} > 1.1$, or reaching a rate of perceived exertion (RPE) > 16 on the Borg scale (Borg, 1982). $\dot{V}\text{O}_{2\text{max}}$ was considered the highest $\dot{V}\text{O}_2$ recorded during the test for successive minute intervals. The same testing protocols were administered during post-testing.

Resting HR data was collected during a RMR testing each subject completed within seven days of their body composition and aerobic capacity test. All resting measurements were completed 36 hours after the last exercise session. Subjects reported to the HSPL for their testing after a full night's rest (≥ 8 hrs) and in a fasted state (≥ 12 hours post prandial). Resting tests were completed between 6:00 and 9:00 a.m. to control for diurnal variation, and pre- and post-testing for each subject was completed at the same time of day. After BW and height were measured, subjects were fitted with a HR monitor strap (Polar, Kempele, Finland) and facemask with a pneumotachometer (MGC diagnostics, St. Paul, Minnesota). A 10-minute period of quiet rest in a supine position then preceded the ensuing RMR measurement. All variables monitored during the resting test were monitored for 35-minutes. Ambient room temperature was maintained at $\sim 21 \pm 1^\circ\text{C}$, lights were dimmed, and noise was kept at a minimum during testing. Subjects were instructed to remain awake but as quiet as possible without distractions from external devices before and throughout the entire session. HR measured during the last 25-minutes of the 35-minutes measurement were used for resting HRV analysis calculations.

Collection of raw HR data was completed using Polar Protrainer 5 software (Polar, Kempele, Finland). Raw data was checked for artifact prior to analysis. Kubios Heart Rate Variability software Version 2 (University of Kupio, Kupio, Finland) was used to analyze of R-R interval time domain, frequency domain, and entropy variability. Default values of $m = 2$ and $r = 0.2 \cdot SD$ were used during SampEn analysis.

Data were analyzed using the SPSS Statistics version 24 (IBM, Armonk, NY). All variables were checked for normality prior to statistical analysis. There was no difference between male and females in regard to the variables measured, so they were considered together as one group. A repeated-measures paired *t*-test analysis was used to examine the effects of the 16-week marathon-training program on all dependent variables.

Results

Table 6: Baseline characteristics of the subjects (Mean \pm SD).

Age (yrs)	20.78 \pm 1.38
Height (m)	1.70 \pm 0.05
Weight (kg)	69.24 \pm 8.60
BMI (kg/m²)	23.79 \pm 2.14
% BF	19.40 \pm 5.39
$\dot{V}O_{2max}$ (ml/kg*min⁻¹)	48.47 \pm 8.35

HR decreased and R-R interval increased significantly during steady-state running at equal work rate from pre- to post-training ($p=0.03$ and $p=0.01$ respectively). The frequency domain measurement SD1 increased from pre- to post-training in steady-state running as well ($p=0.01$). There were no other

significant changes during steady-state running in time domain, frequency domain, or entropy measurements.

Table 7: HRV results during steady-state submaximal running pre- and post-training. (Mean \pm SD)

	Pre-training	Post-training	<i>p</i>-value
HR (bpm)	165.20	156.82	0.03*
RR (ms)	365.58	386.29	0.01*
SDNN (ms)	17.05	21.34	0.08
SD1 (ms)	2.72	3.56	0.01*
SD2 (ms)	23.38	25.79	0.51
SD2/SD1 ratio	9.010	7.601	0.38
SampEn	0.6518	0.6583	0.96

* Statistical significant difference from pre-training to post-training ($p=0.05$)

There were no statistical significant changes in any of the time domain, frequency domain, or entropy measurements of HRV at rest following marathon-training.

Table 8: HRV results during rest pre- and post-training. (Mean \pm SD)

	Pre-training	Post-training	<i>p</i>-value
HR (bpm)	60.00	59.74	0.84
RR (ms)	1046.07	1046.12	0.99
SDNN (ms)	140.57	137.67	0.86
SD1 (ms)	97.15	89.56	0.54
SD2 (ms)	188.26	156.88	0.16
SD2/SD1 ratio	2.060	2.052	0.97
SampEn	1.418	1.481	0.54

Discussion

The significant change in HR and R-R interval during steady-state submaximal running, in addition to the increase in $\dot{V}O_{2\max}$ of these subjects, indicates an overall increase in aerobic fitness was achieved after completing the 16-week marathon-training program. The increase in the SD1 measurement of the Poincare plot suggests an increase in the parasympathetic activity. This is likely a result of adaptation long-term aerobic training as seen in other studies as well (Buchheit & Gindre, 2006; Buchheit, Chovit, Parouty, et al., 2010). This change in a HRV component likely represents a dose-response relationship between training load and HRV as observed in other studies with recreational marathon runners (Manzi, Castagna, et al., 2009). Despite the changes in time domain and frequency domain measurements, there was no change in SampEn R-R variability measurement during submaximal exercise. This result does not match the increased SampEn R-R variability observed by previous research in our lab conducted on a similar group of runners (Brown, 2013). However, Brown (2013) had participants complete the submaximal exercise test at a work rate equal to 65% of current $\dot{V}O_{2\max}$ pace. Conversely, in this study, the steady-state submaximal exercise was kept at the same metabolic equivalents (METs) pre- and post-training. By keeping METs the same pre- to post-training, we were able to observe changes in parasympathetic activity at equal work rates to assess the effect of the exercise intervention.

Measurements of HR and HRV at rest did not show significant change in time domain, frequency domain, or entropy measurements following marathon-training. Previous research with similar results of increased parasympathetic activity during submaximal exercise also demonstrated that an effective tapering phase results in a decrease in HRV at rest, from pre- to post-training (Hug, Heyer, Naef, Buccheit, Wehrlin, & Millet, 2013; Plews et al., 2013). Hug et al. (2013) collected HRV data throughout a 2-week taper period following marathon-training and observed a gradual decrease in the time domain measurement SDNN. The current study collected HRV data only at one time point post-training during the final week of a 2-week taper phase. Thus there may have been a similar decline in HRV at rest from pre- to post-taper that negated significant increases in HRV.

Limitations to the study include having subjects that might have started with higher fitness levels than a normal population. In addition, it would have been useful to have measurements at more time points during training, tapering, and post event. Finally, while HRV measurement and analysis has become more common amongst researchers and exercise scientist, there is no consensus regarding what analysis method provides the most complete picture of ANS response to exercise. When interpreting HRV data, one must consider not only the measurement tool (i.e. time domain, frequency domain, or entropy) but also the physiological state (i.e. resting, submaximal exercise, or maximal exercise).

This study provided insight into the use of several of the HRV measurement tools both during submaximal exercise and at rest in a group of healthy college-aged novice marathon runners. The findings of this study also support the body of research that there is an increase in HRV during submaximal exercise. It is still unclear whether this indicates an increase in PNS and/or decrease in SNS activity. Overall, a more balanced sympathetic tone as been suggested to be favorable for aerobic performance in endurance athletes suggesting that monitoring HRV measurements during endurance training can be useful in predicting an athlete's readiness to perform (Iwasaki, Zhang, Zuckerman, & Levin, 2003; Manzi et al., 2009). Future examination of HRV using all measurement and analysis tools will provide further understanding of ANS changes in response to exercise.

Chapter 7: References

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Chapter 8: Appendices

8.1 Consent Form

CONSENT FORM **Physiological Adaptations To Marathon Training**

You are invited to participate in a research study which will document the physiological and physical adaptations to marathon running. You were selected as a possible participant because of your enrollment in PE 1262: Marathon Training at the University of Minnesota. We ask that you read this form and ask any questions you may have before agreeing to be in the study.

The instructor of PE 1262: Marathon Training will have no knowledge of your choice to be included in the research portion of the class, and therefore your choice to participate in the research will have no bearing on your grade in the class.

This study is being conducted by lead investigator Stacy Ingraham, Ph.D., a faculty member in the School of Kinesiology at the University of Minnesota. The research is funded by the Human and Sport Performance Laboratory.

Study Purpose

The primary objective of this study is to document adaptations that are associated with marathon training in novice, recreational runners. Specifically, the research aims to further understand the relationships between training and running performance, metabolic adaptations, psychology motivations, and core stability/balance.

Study Procedures

Please check the boxes below if agree to allow the information collected for course purposes to be used in this research study:

- ☐ **Pre-course questionnaire** – Your answers on the pre-course questionnaire (completed online) may be used for research purposes.
- ☐ **Two mile time trial** – Finishing time and average speed maintained during the time trial may be used for research purposes.

- ☐ **Daily Running Journal data** – Daily record of running mileage, minutes, modality of training, RPE, mood states, sleep hours, injury reports. This information allows for stratification of the data for research.

Test Session I (Approximately 120 minutes)

- ☐ **Psychological assessments** - Your answers on questionnaires covering mood state, goal setting, athletic identity, motivation, and physical self perception may be used for research purposes.
- ☐ **Height, body weight, and body composition** - This information is collected for course purposes; please check the box if you will allow this data to be used to research. Your height and weight will be measured using a digital scale. Body composition will be assessed using an underwater weighing technique which will require you to be completely submerged underwater for brief periods of time.
- ☐ **Treadmill test** - This information is collection for course purposes; Please check the box if you will allow the information collected to be used for research. You will be asked to exercise on a running treadmill to fatigue. A facemask which monitors exhaled air will be worn for the duration of the test. Additionally, you may also have reflective markers applied to you torso and legs such that each segment of your lower extremity is monitored. These reflective markers are captured by a 3D motion tracking system. No images of you will exist, but rather, just the markers. The testing protocol consists of stages in which work rate is progressively increased through increased treadmill velocity and/or treadmill incline. Spotters will be near to assist as you approach fatigue.

Test session II (Approximately 60 minutes)

These tests are optional. Please check the box if you understand the testing protocols, would like to perform the tests, and will allow the information collected to be used for research.

- ☐ **Resting Metabolic Rate test** – You will be asked to arrive to the lab rested and fasted for at least 8hrs. You will record your diet for the 3 – days prior to the testing session and bring a food journal log to the

testing period. During the test you will be asked to rest in a supine position while wearing a facemask that monitors exhaled and inhaled air and a heart rate monitor. You will rest in this supine position for 40 minutes. During this rest you will be asked to not fall asleep and will not be able to use your smart phone, tablet, or computer.

Test session III (Approximately 30 minutes)

These tests are optional. Please check the box if you understand the testing protocols, would like to perform the tests, and will allow the information collected to be used for research.

☐

Vertical Jump Test – You will be asked to jump as high as possible using a standard vertical jump testing procedure.

Wingate Anaerobic Test – This test occurs on an exercise cycle. A five minute standardized warm-up protocol will be explained to you prior to the test. During the test, you will be asked to pedal at a maximal intensity against resistance on the exercise cycle for a 30 second duration.

Risk of Study Participation

During testing which involves moderate to high physical exertion such as treadmill running and cycling, you will perform until fatigue. At that point, you may end the testing by grabbing the treadmill handles, straddling the treadmill, or hitting the manual treadmill stop button. Throughout testing, first aid and CPR/AED certified investigators will be monitoring you. These tests represent what may be a change from normal physical activity levels and in rare cases may result in injury or in extremely rare cases, death.

Benefits of Study Participation

You will receive the results of the laboratory testing which will aid in the educational experience (as associated with the PE 1262: Marathon Training course) in the marathon training process. There are no direct health benefits to participating in the laboratory tests.

Study Costs/Compensation

You will not incur any costs during this study, nor will you be compensated monetarily for your time.

Research Related Injury

In the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment and follow-up care as needed. Care for such injuries will be billed in the ordinary manner to you or your insurance company. If you think that you have suffered a research related injury, let the study physicians know right away.

Confidentiality

The records of this study will be kept private. In any publications or presentations, we will not include any information that will make it possible to identify you as a subject. Your record for the study may, however, be reviewed by departments at the University with appropriate regulatory oversight. To these extents, confidentiality is not absolute.

Voluntary Nature of the Study

Participation in this study is voluntary. Your decision whether or not to participate in this study will not affect your current or future relations with the University of Minnesota, the Human and Sport Performance Lab, or your final grade in PE 1262: Marathon Training. If you decide to participate, you are free to withdraw at any time without affecting those relationships.

Contacts and Questions

The researcher conducting this study is Stacy Ingraham, Ph.D., a faculty member in the School of Kinesiology at the University of Minnesota. You may ask any questions you have now, or if you have questions later, **you are encouraged to** contact them at:

- Stacy Ingraham: Office phone – 612.626.0067

If you have any questions or concerns regarding the study and would like to talk to someone other than the researcher(s), you are encouraged to contact the Fairview Research Help-line at telephone number 612-672-7692 or toll free at 866-508-6951. You may also contact this office in writing or in person at University of Minnesota Medical Center, Fairview-Riverside Campus, 2200 Riverside Avenue, Minneapolis, MN 55454.

You will be given a copy of this form to keep for your records.

Statement of Consent

I have read the above information. I have asked questions and have received answers. I consent to participate in the study.

Name of Subject (please print legibly): -

Signature of Subject:

Date: _____

Signature of Investigator:

Date: _____

8.2 Pre-testing Instructions

UNIVERSITY OF MINNESOTA

Dear _Marathon Class Runner_____

Your lab testing time is coming up shortly. Please be prompt and prepare for this test as indicated below. Please plan on this appointment lasting between 1 hour 45 minutes and 2 hours 15 minutes.

In order to ensure the utmost in accuracy we ask that you comply with the following:

Pre-Test Instructions:

Be sure that you are rested. If you exercise the day before the test, be sure it is of light to moderate intensity an relatively short duration. You should not exercise within 12 hours of your test.

You should not have eaten within 3-4 hours of the test.

Avoid alcohol, caffeine, and tobacco within 8 hours of the test.

Be sure you are adequately hydrated. Drink adequate amount of water during the hours before the test.

Clothing to bring with you for your testing:

- Running shoes
- Swim suit and towel
- Spandex running shorts (tight running shorts are needed for the biomechanic assessment)
- Running top (Women please make sure to be wearing a jog bra)

Additional Preparation and Instructions:

Upon your arrival, you will be given a medical history questionnaire and a consent form. Please be sure to have all necessary information available such any pertinent medical information.

If you must cancel or reschedule your test, please do so at least 48 hours in advance. (contact Greg Rhodes, rhod0048@umn.edu)

I have read, understand, and agree to the above guidelines and policies.

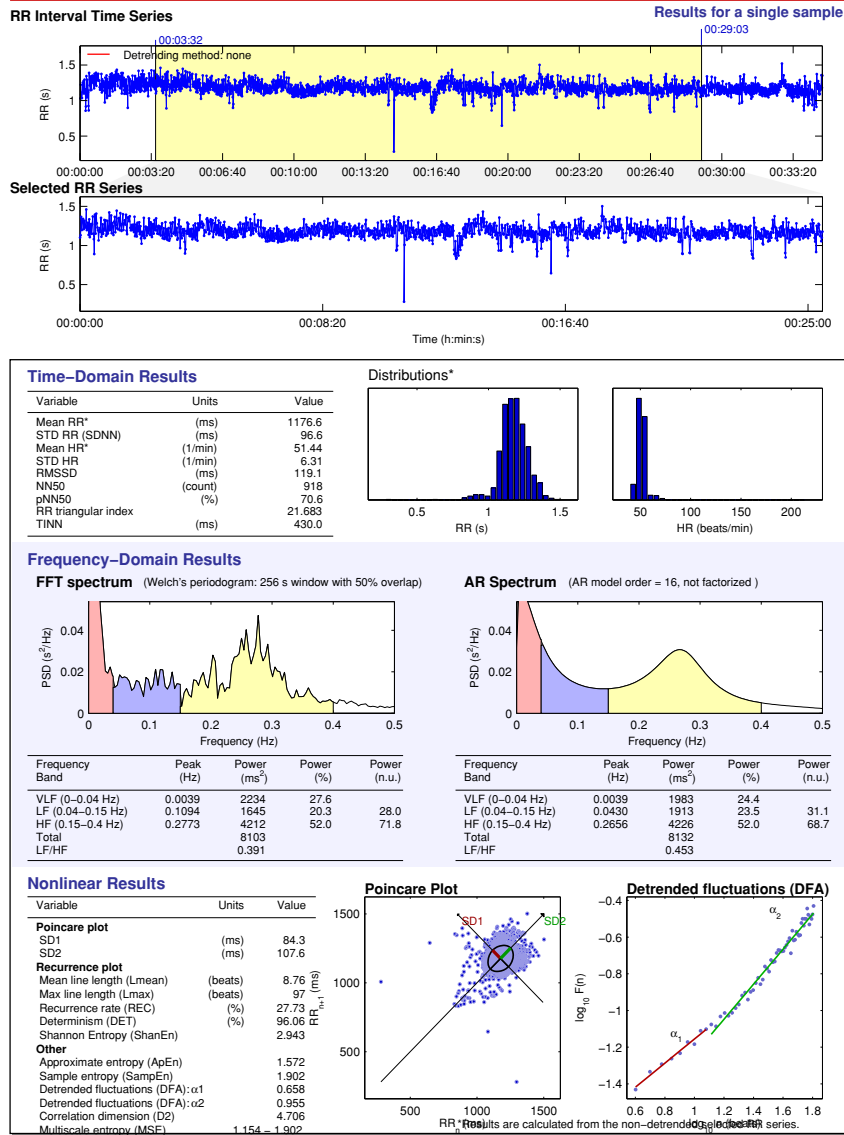
Signature _____ Date: _____

8.3 Sample Kubios files

HRV Analysis Results

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16-Jul-2016 09:13:54
Greg Rhodes
School of Kinesiology, University of Minnesota

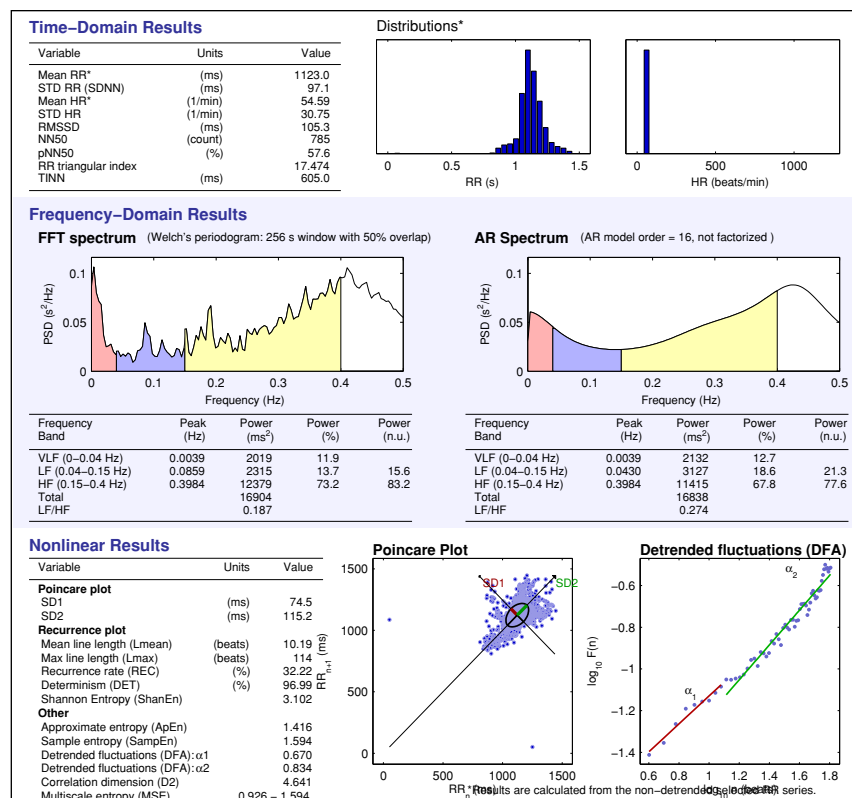
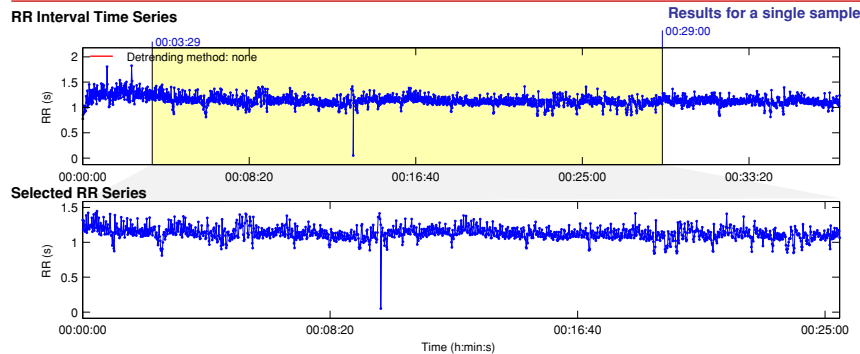
Kubios HRV, 2.2
Department of Applied Physics
University of Eastern Finland, Kuopio, Finland

Figure 2 Example pre-training resting HRV Kubios data analysis file

HRV Analysis Results

241_post.txt - xx/xx/xx - xx:xx:xx

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16-Jul-2016 09:37:00
Greg Rhodes
School of Kinesiology, University of Minnesota

Kubios HRV, 2.2
Department of Applied Physics
University of Eastern Finland, Kuopio, Finland

Figure 3 Example post-training resting HRV Kubios data analysis file

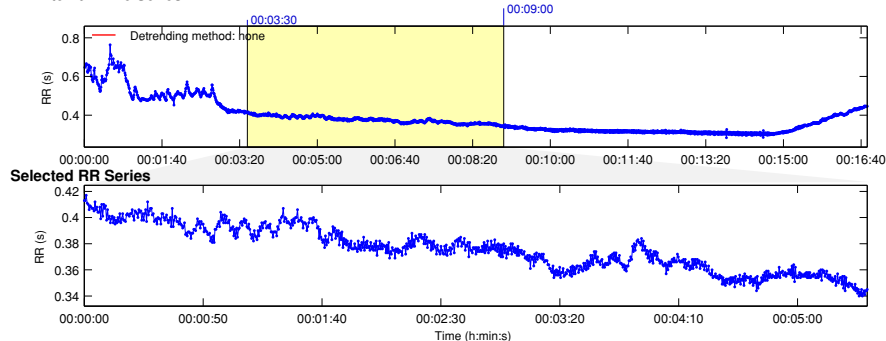
HRV Analysis Results

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RR Interval Time Series

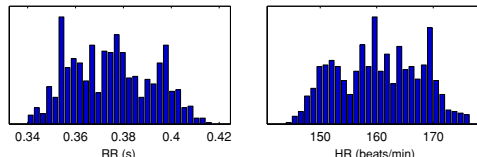
Results for a single sample



Time-Domain Results

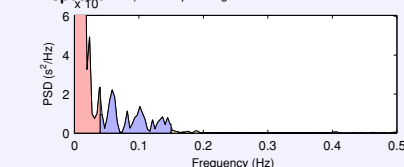
Variable	Units	Value
Mean RR*	(ms)	375.1
STD RR (SDNN)	(ms)	17.1
Mean HR*	(1/min)	160.28
STD HR	(1/min)	7.27
RMSSD	(ms)	4.8
NN50	(count)	0
pNN50	(%)	0.0
RR triangular index		5.599
TINN	(ms)	75.0

Distributions*



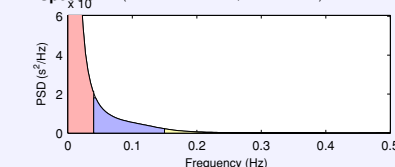
Frequency-Domain Results

FFT spectrum (Welch's periodogram: 256 s window with 50% overlap)



Frequency Band	Peak (Hz)	Power (ms ²)	Power (%)	Power (n.u.)
VLF (0-0.04 Hz)	0.0039	86	90.9	
LF (0.04-0.15 Hz)	0.0586	8	8.6	93.9
HF (0.15-0.4 Hz)	0.1523	1	0.5	6.0
Total		95		
LF/HF		15.591		

AR Spectrum (AR model order = 16, not factorized)

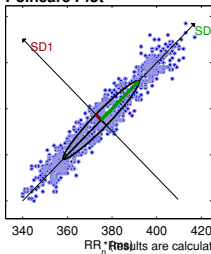


Frequency Band	Peak (Hz)	Power (ms ²)	Power (%)	Power (n.u.)
VLF (0-0.04 Hz)	0.0000	225	96.3	
LF (0.04-0.15 Hz)	0.0430	8	3.3	88.8
HF (0.15-0.4 Hz)	0.1523	1	0.4	11.2
Total		234		
LF/HF		7.950		

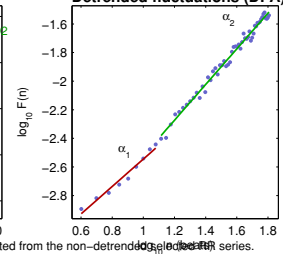
Nonlinear Results

Variable	Units	Value
Poincare plot		
SD1	(ms)	3.4
SD2	(ms)	23.9
Recurrence plot		
Mean line length (Lmean)	(beats)	62.83
Max line length (Lmax)	(beats)	869
Recurrence rate (REC)	(%)	48.69
Determinism (DET)	(%)	99.80
Shannon Entropy (ShanEn)		4.712
Other		
Approximate entropy (ApEn)		0.591
Sample entropy (SampEn)		0.540
Detrended fluctuations (DFA): α_1		0.960
Detrended fluctuations (DFA): α_2		1.248
Correlation dimension (D2)		0.208
Multiscale entropy (MSE)		0.361 - 0.967

Poincare Plot



Detrended fluctuations (DFA)



16-Jul-2016 01:07:01
Greg Rhodes
School of Kinesiology, University of Minnesota

Kubios HRV, 2.2
Department of Applied Physics
University of Eastern Finland, Kuopio, Finland

Figure 4 Example pre-training submaximal steady-state running HRV Kubios data analysis file

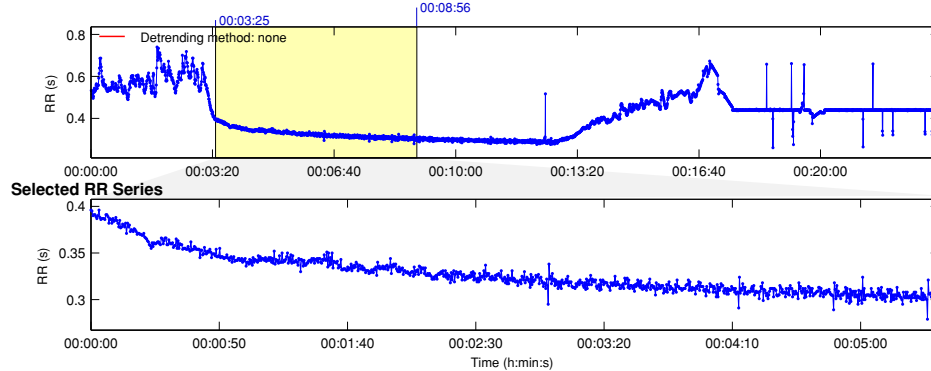
HRV Analysis Results

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RR Interval Time Series

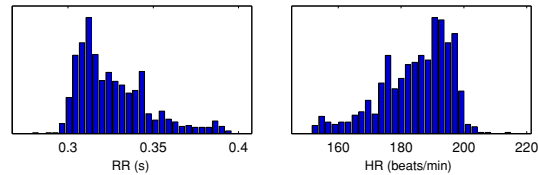
Results for a single sample



Time-Domain Results

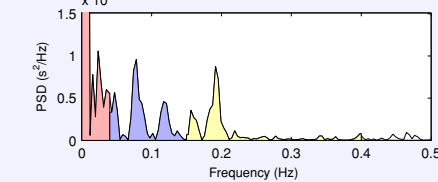
Variable	Units	Value
Mean RR*	(ms)	326.4
STD RR (SDNN)	(ms)	20.9
Mean HR*	(1/min)	184.53
STD HR	(1/min)	11.16
RMSSD	(ms)	6.0
NN50	(count)	0
pNN50	(%)	0.0
RR triangular index		4.673
TINN	(ms)	85.0

Distributions*



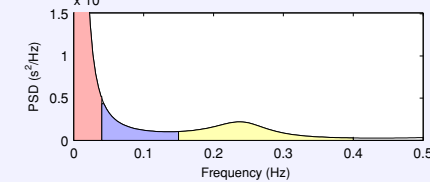
Frequency-Domain Results

FFT spectrum (Welch's periodogram: 256 s window with 50% overlap)



Frequency Band	Peak (Hz)	Power (ms ²)	Power (%)	Power (n.u.)
VLF (0-0.04 Hz)	0.0039	69	93.5	
LF (0.04-0.15 Hz)	0.0781	3	3.7	56.5
HF (0.15-0.4 Hz)	0.1914	2	2.8	43.3
Total		74		
LF/HF		1.305		

AR Spectrum (AR model order = 16, not factorized)

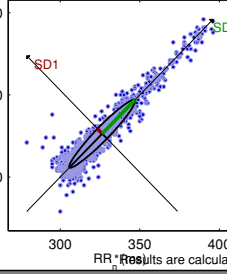


Frequency Band	Peak (Hz)	Power (ms ²)	Power (%)	Power (n.u.)
VLF (0-0.04 Hz)	0.0000	1910	99.8	
LF (0.04-0.15 Hz)	0.0430	2	0.1	40.0
HF (0.15-0.4 Hz)	0.2383	3	0.1	59.9
Total		1915		
LF/HF		0.669		

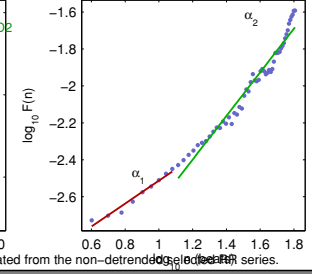
Nonlinear Results

Variable	Units	Value
Poincare plot		
SD1	(ms)	4.3
SD2	(ms)	29.2
Recurrence plot		
Mean line length (Lmean)	(beats)	123.14
Max line length (Lmax)	(beats)	990
Recurrence rate (REC)	(%)	56.78
Determinism (DET)	(%)	99.68
Shannon Entropy (ShanEn)		4.540
Other		
Approximate entropy (ApEn)		0.609
Sample entropy (SampEn)		0.547
Detrended fluctuations (DFA): α_1		0.623
Detrended fluctuations (DFA): α_2		1.182
Correlation dimension (D2)		0.342
Multiscale entropy (MSE)		0.120 - 0.547

Poincare Plot



Detrended fluctuations (DFA)



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Figure 5 Example post-training submaximal steady-state running HRV Kubios data analysis file